

10759-31

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/JP05/005613

International filing date: 18 March 2005 (18.03.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/555,789
Filing date: 23 March 2004 (23.03.2004)

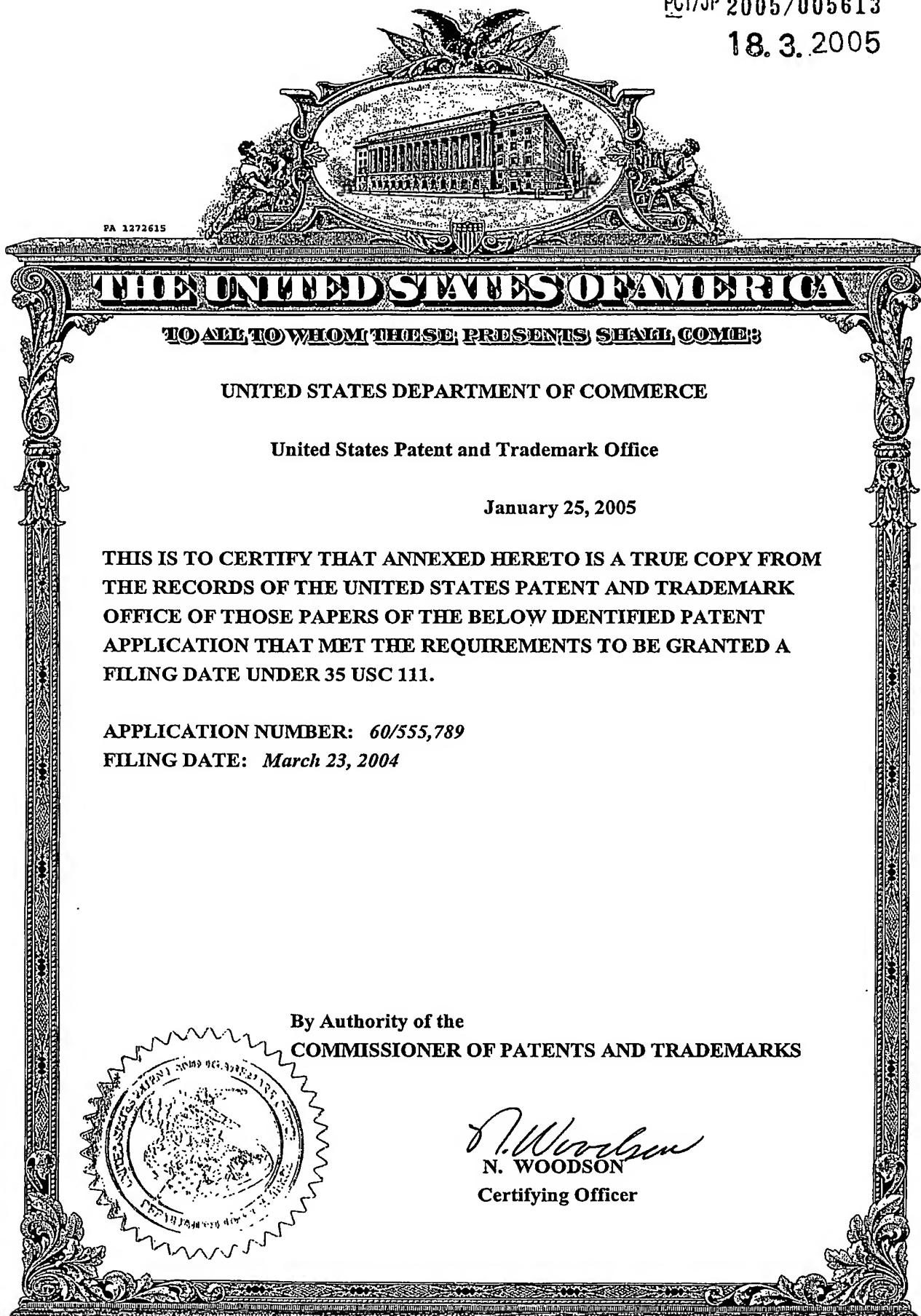
Date of receipt at the International Bureau: 07 April 2005 (07.04.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

PCT/JP 2005/005613
18.3.2005



PROVISIONAL APPLICATION FOR PATENT COVER SHEET

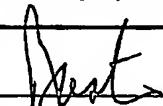
This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 330859025 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Yusuke	Nakamura	Kanagawa, Japan
Yataro	Dalgo	Kanagawa, Japan
<input checked="" type="checkbox"/> Additional inventors are being named on the 1 separately numbered sheets attached hereto		
TITLE OF THE INVENTION (500 characters max)		
METHOD FOR DIAGNOSING NON-SMALL CELL LUNG CANCER		
Direct all correspondence to: CORRESPONDENCE ADDRESS		
<input checked="" type="checkbox"/> Customer Number 20350		
OR		
<input type="checkbox"/> Firm or Individual Name		
Address		
Address		
City	State	ZIP
Country	Telephone	Fax
ENCLOSED APPLICATION PARTS (check all that apply)		
<input checked="" type="checkbox"/> Specification Number of Pages 74 <input type="checkbox"/> CD(s), Number _____		
<input checked="" type="checkbox"/> Drawing(s) Number of Sheets 11 <input checked="" type="checkbox"/> Other (specify) Sequence Listing (33pp)		
<input checked="" type="checkbox"/> Application Data Sheet. See 37 CFR 1.76		
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT		
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. <input type="checkbox"/> A check or money order is enclosed to cover the filing fees <input checked="" type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 20-1430 <input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.		FILING FEE Amount (\$) 160
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.		
<input checked="" type="checkbox"/> No. <input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____		

Respectfully submitted,

[Page 1 of 2]

Date March 23, 2004SIGNATURE REGISTRATION NO. 34,774TYPED or PRINTED NAME Kevin Bastian

(if appropriate)

TELEPHONE 415-576-0200Docket Number: 082368-000500US

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

60172423 v1

PROVISIONAL APPLICATION COVER SHEET*Additional Page*

PTO/SB/16 (08-03)

Docket Number 082368-000500US		
INVENTOR(S)/APPLICANT(S)		
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Shulchi	Nakatsuru	Saitama, Japan

[Page 2 of 2]

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.
60172423 v1

Attorney Docket No.: 082368-000500US
Client Reference No.: ONC-PRV0401/US

PROVISIONAL

PATENT APPLICATION

METHOD FOR DIAGNOSING NON-SMALL CELL LUNG CANCER

Inventor(s): Yusuke Nakamura, a citizen of Japan, residing at
Kanagawa, Japan

Yataro Daigo, a citizen of Japan, residing at
Kanagawa, Japan

Shuichi Nakatsuru, a citizen of Japan, residing at
Saitama, Japan

Assignee:

Entity: Large

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200

METHOD FOR DIAGNOSING NON-SMALL CELL LUNG CANCER

FIELD OF THE INVENTION

The present invention relates to the field of biological science, more specifically to the field of cancer research. In particular, the invention relates to methods of diagnosing non-small cell lung cancers using genes, KIF11, GHSR1b, and NTSR1, that show elevated expression in such cancerous cells.

BACKGROUND OF THE INVENTION

10 Lung cancer is one of the most commonly fatal human tumors. Many genetic alterations associated with the development and progression of lung cancer has been reported. Although genetic changes can aid prognostic efforts and predictions of metastatic risk or response to certain treatments, information about a single or a limited number of molecular markers generally fails to provide satisfactory results for clinical diagnosis of non-small cell lung cancer (NSCLC)(Mitsudomi et al., *Clin Cancer Res* 6: 4055-63 (2000); Niklinski et al., *Lung Cancer*, 34 Suppl 2: S53-8 (2001); Watine, *Bmj* 320: 379-80 (2000)). NSCLC is by far the most common form, accounting for nearly 80% of lung tumors (Society, A.C. *Cancer Facts and Figures 2001* (2001)). The overall 10-year survival rate remains as low as 10% despite recent advances in multi-modality therapy, because the majority of NSCLCs are not diagnosed until advanced stages (Fry, W.A., Phillips, J.L., and Menck, H.R. "Ten-year survey of lung cancer treatment and survival in hospitals in the United States: a national cancer data base report", *Cancer* 86: 1867-76 (1999)). Although chemotherapy regimens based on platinum are considered the reference standards for treatment of NSCLC, those drugs are able to extend survival of patients with advanced NSCLC only about six weeks ("Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials." Non-small Cell Lung Cancer Collaborative Group, *Bmj*. 311: 899-909 (1995)). Numerous targeted therapies are being investigated for this disease, including tyrosine kinase inhibitors, but so far promising results have been achieved in only a limited number of patients and some recipients suffer severe adverse reactions (Kris M.N.R., Herbst R.S. "A phase II trial of ZD1839 ('Iressa') in advanced non-small cell lung cancer (NSCLC) patients who had failed platinum- and docetaxel-based regimens (IDEAL 2).", *Proc. Am. Soc. Clin. Oncol.* 21: 292a(A1166) (2002)).

35 Many genetic alterations associated with development and progression of lung cancer have been reported, but the precise molecular mechanisms remain unclear (Sozzi, G. "Molecular biology of lung cancer." *Eur. J. Cancer* 37: 63-73 (2001)). Over the last decade newly

developed cytotoxic agents including paclitaxel, docetaxel, gemcitabine, and vinorelbine have emerged to offer multiple therapeutic choices for patients with advanced NSCLC; however, each of the new regimens can provide only modest survival benefits compared with cisplatin-based therapies (Schiller, J.H. et al. "Comparison of four chemotherapy regimens for 5 advanced non-small-cell lung cancer." *N. Engl. J. Med.* 346: 92-98 (2002); Kelly, K. et al. "Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non-small-cell lung cancer: a Southwest Oncology Group trial." *J. Clin. Oncol.* 19: 3210-3218 (2001)). Hence, new therapeutic strategies, such as development of molecular-targeted agents, are eagerly awaited by clinicians.

10 Systematic analysis of expression levels of thousands of genes on cDNA microarrays is an effective approach to identifying unknown molecules involved in pathways of carcinogenesis (Kikuchi, T. et al. "Expression profiles of non-small cell lung cancers on cDNA microarrays: Identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs." *Oncogene* 22: 2192-2205 (2003); Kakiuchi, S. et al. "Genome-wide analysis of 15 organ-preferential metastasis of human small cell lung cancer in mice." *Mol. Cancer Res.* 1: 485-499 (2003); Zembutsu, H. et al. "Gene-expression profiles of human tumor xenografts in nude mice treated orally with the EGFR tyrosine kinase inhibitor ZD1839." *Int. J. Oncol.* 23: 29-39 (2003); Suzuki, C., Daigo, Y., Kikuchi, T., Katagiri, T. & Nakamura, Y. "Identification of COX17 as a therapeutic target for non-small cell lung cancer." *Cancer Res.* 63: 7038-7041 (2003)) and can reveal candidate targets for development of novel anti-cancer drugs and tumor markers. To isolate novel molecular targets for diagnosis, treatment and prevention of NSCLC, the present inventors prepared have been pure populations of tumor cells were prepared from 37 cancer tissues by laser-capture microdissection and analyzed genome-wide expression profiles of NSCLC cells on a cDNA microarray containing 23,040 genes (Kikuchi, T. et al. "Expression 20 profiles of non-small cell lung cancers on cDNA microarrays: Identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs." *Oncogene* 22: 2192-2205 (2003)). In the course of those experiments, KOC1 (GenBank Accession No.NM_006547) and neuromedin U (NMU; GenBank Accession No. NM_006681) were identified as genes that were frequently over-expressed in lung tumors and indispensable for 25 growth of NSCLC cells.

30 The phenomenon of mRNA localization has been reported in oocytes and developing embryos of *Drosophila* and *Xenopus* and in somatic cells such as fibroblasts and neurons (King, M.L., Zhou, Y., Bubunenko, M. "Polarizing genetic information in the egg: RNA localization in the frog oocyte." *Bioessays* 21: 546-557 (1999); Mowry, K.L., Cote, C.A. "RNA sorting in 35 *Xenopus* oocytes and embryos." *FASEB J.* 13: 435-445 (1999); Lasko, P. "The *drosophila*

melanogaster genome: translation factors and RNA binding proteins." *J. Cell Biol.* 150: F51-56 (2000); Steward, O. "mRNA localization in neurons: a multipurpose mechanism?" *Neuron* 18: 9-12 (1997)). ACTB mRNA is localized at the leading lamellae of chicken embryo fibroblasts (CEFs)(Lawrence, J.B., Singer, R.H. "Intracellular localization of messenger RNAs for cytoskeletal proteins." *Cell* 45: 407-415 (1986)) and at the growth cone of developing neurons (Bassell, G.J., Zhang, H., Byrd, A.L., Femino, A.M., Singer, R.H., Taneja, K.L., Lifshitz, L.M., Herman, I.M., Kosik, K.S. "Sorting of beta-actin mRNA and protein to neurites and growth cones in culture." *J. Neurosci.* 18: 251-265 (1998)). The localization of ACTB mRNA is dependent of the zipcode, a cis-acting element located in the 3' UTR of the mRNA (Kislauksis, 5 E.H., Li, Z., Singer, R.H., Taneja, K.L. "Isoform-specific 3'-untranslated sequences sort alpha-cardiac and beta-cytoplasmic actin messenger RNAs to different cytoplasmic compartments." *J. Cell Biol.* 123: 165-172 (1993)). The trans-acting factor, zipcode binding protein 1 (ZBP1), was affinity purified with the zipcode of ACTB mRNA (Ross, A.F., Oleynikov, Y., Kislauksis, E.H., Taneja, K.L., Singer, R.H. "Characterization of a beta-actin 10 mRNA zipcode-binding protein." *Mol. Cell Biol.* 17, 2158-2165 (1997)). After the identification of ZBP1, additional homologues were identified in a wide range of organisms including Xenopus, Drosophila, human, and mouse (Mueller-Pillasch, F., Lacher, U., Wallrapp, C., Micha, A., Zimmerhackl, F., Hameister, H., Varga, G., Friess, H., Buchler, M., Beger, H.G., Vila, M.R., Adler, G., Gress, T.M. "Cloning of a gene highly overexpressed in cancer coding for 15 a novel KH-domain containing protein." *Oncogene* 14: 2729-2733 (1997); Deshler, J.O., Hightett, M.I., Schnapp, B.J. "Localization of Xenopus Vg1 mRNA by Vera protein and the endoplasmic reticulum." *Science* 276: 1128-1131 (1997); Doyle, G.A., Betz, N.A., Leeds, P.F., Fleisig, A.J., Prokipcak, R.D., Ross, J. "The c-myc coding region determinant-binding protein: a member of a family of KH domain RNA-binding proteins." *Nucleic Acids Res.* 26: 20 5036-5044 (1998)). ZBP1 family members are expressed in germ embryonic fibroblasts and in several types of cancer (Mueller-Pillasch, F., Lacher, U., Wallrapp, C., Micha, A., Zimmerhackl, F., Hameister, H., Varga, G., Friess, H., Buchler, M., Beger, H.G., Vila, M.R., Adler, G., Gress, T.M. "Cloning of a gene highly overexpressed in cancer coding for a novel KH-domain containing protein." *Oncogene* 14: 2729-2733 (1997); Mueller, F., Bommer, M., 25 Lacher, U., Ruhland, C., Stagge, V., Adler, G., Gress, T.M., Seufferlein, T. "KOC is a novel molecular indicator of malignancy." *Br. J. Cancer* 88; 699-701 (2003)). ZBP1-like proteins contain two RNA-recognition motifs (RRMs) at the NH2-terminal part of the protein and four hnRNP K homology (KH) domains at the COOH-terminal end.

KOC1 (alias IGF-II mRNA-binding protein 3: IMP-3) is one of the IMPs (IMP-1, IMP-2, 30 and IMP-3), which belong to the ZBP1 family members and exhibit multiple attachments to

IGF-II leader 3 mRNA and the reciprocally imprinted H19 RNA (Mueller-Pillasch, F., Lacher, U., Wallrapp, C., Micha, A., Zimmerhackl, F., Hameister, H., Varga, G., Friess, H., Buchler, M., Beger, H.G., Vila, M.R., Adler, G., Gress, T.M. "Cloning of a gene highly overexpressed in cancer coding for a novel KH-domain containing protein." *Oncogene* 14: 2729-2733 (1997)).

5 Although KOC1 was initially reported to be over-expressed in pancreatic cancer (Mueller-Pillasch, F., Lacher, U., Wallrapp, C., Micha, A., Zimmerhackl, F., Hameister, H., Varga, G., Friess, H., Buchler, M., Beger, H.G., Vila, M.R., Adler, G., Gress, T.M. "Cloning of a gene highly overexpressed in cancer coding for a novel KH-domain containing protein." *Oncogene* 14: 2729-2733 (1997); Mueller, F., Bommer, M., Lacher, U., Ruhland, C., Stagge, V.,

10 Adler, G., Gress, T.M., Seufferlein, T. "KOC is a novel molecular indicator of malignancy." *Br. J. Cancer* 88: 699-701 (2003)), its precise function in cancer cells or even in normal mammalian somatic cells remains unclear.

KOC1 is orthologous to the *Xenopus* Vg1 RNA-binding protein (Vg1RBP/Vera), which mediates the localization of Vg1 mRNA to the vegetal pole of the oocyte during oocyte maturation, and IMP-1 is orthologous to the ZBP1. IMP is mainly located at the cytoplasm and its cellular distribution ranges from a distinct concentration in perinuclear regions and lamellipodia to a completely delocalized pattern. H19 RNA co-localized with IMP, and removal of the high-affinity attachment site led to delocalization of the truncated RNA (Runge, S., Nielsen, F.C., Nielsen, J., Lykke-Andersen, J., Wewer, U.M., Christiansen, J.H. "19 RNA binds four molecules of insulin-like growth factor II mRNA-binding protein." *J. Biol. Chem.* 275: 29562-29569 (2000)), suggesting that IMPs are involved in cytoplasmic trafficking of RNA. IMP-1 was able to associate with microtubules (Nielsen, F.C., Nielsen, J., Kristensen, M.A., Koch, G., Christiansen, J. "Cytoplasmic trafficking of IGF-II mRNA-binding protein by conserved KH domains." *J. Cell Sci.* 115: 2087-2097 (2002); Havin, L., Git, A., Elisha, Z.,

25 Oberman, F., Yaniv, K., Schwartz, S.P., Standart, N., Yisraeli, J.K. "RNA-binding protein conserved in both microtubule- and microfilament-based RNA localization." *Genes Dev.* 12: 1593-1598 (1998)), and is likely to involve a motor protein such as kinesin, myosin, and dyenin. On the other hand, Oskar mRNA localization to the posterior pole requires Kinésin I (Palacios, I.M., St. Johnston D. "Kinesin light chain-independent function of the Kinesin heavy chain in

30 cytoplasmic streaming and posterior localisation in the *Drosophila* oocyte." *Development* 129: 5473-5485 (2002); Brendza, R.P., Serbus, L.R., Duffy, J.B., Saxton, W.M. "A function for kinesin I in the posterior transport of oskar mRNA and Staufen protein." *Science* 289: 2120-2102 (2000)).

KIF11 (alias EG5) is a member of kinesin family, and plays a role in establishing and/or determining the stability of specific microtubule arrays that form during cell division. This role

may encompass the ability of KIF11 to influence the distribution of other protein components associated with cell division (Whitehead, C.M., Rattner, J.B. "Expanding the role of HsEg5 within the mitotic and post-mitotic phases of the cell cycle." *J. Cell Sci.* 111: 2551-2561 (1998); Mayer, T.U., Kapoor, T.M., Haggarty, S.J., King, R.W., Schreiber, S.L., Mitchison, T.J. "Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen." *Science* 286: 971-974 (1999)).

5 NMU is a neuropeptide that was first isolated from porcine spinal cord. It has potent activity on smooth muscles (Minamino, N., Kangawa, K. & Matsuo, H. "Neuromedin U-8 and U-25: novel uterus stimulating and hypertensive peptides identified in porcine spinal cord." *Biochem. Biophys. Res. Commun.* 130: 1078-1085 (1985); Domin, J., Ghatei, M.A., Chohan, P. & Bloom, S.R. "Characterization of neuromedin U-like immunoreactivity in rat, porcine, guinea-pig and human tissue extracts using a specific radioimmunoassay." *Biochem. Biophys. Res. Commun.* 140: 1127-1134 (1986); Conlon, J.M. et al. "Primary structure of neuromedin U from the rat." *J. Neurochem.* 51: 988-991 (1988); Minamino, N., Kangawa, K., Honzawa, M. & Matsuo, H. "Isolation and structural determination of rat neuromedin U." *Biochem. Biophys. Res. Commun.* 156: 355-360 (1988); Domin, J. et al. "The distribution, purification, and pharmacological action of an amphibian neuromedin U." *J. Biol. Chem.* 264: 20881-20885 (1989), O'Harte, F. et al. "Primary structure and pharmacological activity of a nonapeptide related to neuromedin U isolated from chicken intestine." *Peptides* 12: 809-812 (1991); Kage, R., O'Harte, F., Thim, L. & Conlon, J.M. "Rabbit neuromedin U-25: lack of conservation of a posttranslational processing site." *Regul. Pept.* 33: 191-198 (1991); Austin, C. et al. "Distribution and developmental pattern of neuromedin U expression in the rat gastrointestinal tract." *J. Mol. Endocrinol.* 12: 257-263 (1994); Fujii, R. et al. "Identification of neuromedin U as the cognate ligand of the orphan G protein-coupled receptor FM-3." *J. Biol. Chem.* 275: 21068-21074 (2000)), and in mammalian species NMU is distributed predominantly in the gastrointestinal tract and central nervous system (Howard, A.D. et al. "Identification of receptors for neuromedin U and its role in feeding." *Nature* 406: 70-74 (2000); Funes, S. et al. "Cloning and characterization of murine neuromedin U receptors." *Peptides* 23: 1607-1615 (2002)). Peripheral activities of NMU include stimulation of smooth muscle, elevation of 10 blood pressure, alternation of ion transport in the gut, and regulation of feeding (Minamino, N., Kangawa, K. & Matsuo, H. "Neuromedin U-8 and U-25: novel uterus stimulating and hypertensive peptides identified in porcine spinal cord." *Biochem. Biophys. Res. Commun.* 130: 1078-1085 (1985)); however, the role of NMU during carcinogenesis has not been addressed. Neuropeptides function peripherally as paracrine and autocrine factors to regulate 15 diverse physiologic processes and act as neurotransmitters or neuromodulators in the nervous system.

system. In general, receptors that mediate signaling by binding neuropeptides are members of the superfamily of G protein-coupled receptors (GPCRs) having seven transmembrane-spanning domains. Two known receptors for NMU, NMU1R and NMU2R, show a high degree of homology to other neuropeptide receptors such as GHSR and NTSR1, for which the corresponding known ligands are Ghrelin (GHRL) and neuropeptides (NTS), respectively. NMU1R (FM3/GPR66) and NMU2R (FM4) have seven predicted alpha-helical transmembrane domains containing highly conserved motifs, as do other members of the rhodopsin GPCR family (Fujii, R. et al. "Identification of neuromedin U as the cognate ligand of the orphan G protein-coupled receptor FM-3." *J. Biol. Chem.* 275: 21068-21074 (2000); Howard, A.D. et al. "Identification of receptors for neuromedin U and its role in feeding." *Nature* 406: 70-74 (2000); Funes, S. et al. "Cloning and characterization of murine neuromedin U receptors." *Peptides* 23: 1607-1615 (2002)).

A C-terminal asparaginamide structure and the C-terminal heptapeptide core of NMU protein are essential for its contractile activity in smooth-muscle cells (Westfall, T.D. et al. "Characterization of neuromedin U effects in canine smooth muscle." *J. Pharmacol. Exp. Ther.* 301: 987-992 (2002); Austin, C., Nandha, K.A., Meleagros, L. & Bloom, S.R. "Cloning and characterization of the cDNA encoding the human neuromedin U precursor: NMU expression in the human gastrointestinal tract." *J. Mol. Endocrinol.* 14: 157-169 (1995)). Recent studies have contributed evidence that NMU acts at the hypothalamic level to inhibit food intake; therefore this protein might be a physiological regulator of feeding and body weight (Howard, A.D. et al. "Identification of receptors for neuromedin U and its role in feeding." *Nature* 406: 70-74 (2000); Maggi, C.A. et al. "Motor response of the human isolated small intestine and urinary bladder to porcine neuromedin U-8." *Br. J. Pharmacol.* 99: 186-188 (1990); Wren, A.M. et al. "Hypothalamic actions of neuromedin U." *Endocrinology* 143: 227-234 (2002); Ivanov, T.R., Lawrence, C.B., Stanley, P.J. & Luckman, S.M. "Evaluation of neuromedin U actions in energy homeostasis and pituitary function." *Endocrinology* 143: 3813-3821 (2002)). However, so far no reports have suggested involvement of NMU over-expression in carcinogenesis.

Studies designed to reveal mechanisms of carcinogenesis have already facilitated identification of molecular targets for anti-tumor agents. For example, inhibitors of farnesyltransferase (FTIs) which were originally developed to inhibit the growth-signaling pathway related to Ras, whose activation depends on posttranslational farnesylation, has been effective in treating Ras-dependent tumors in animal models (He et al., *Cell* 99:335-45 (1999)). Clinical trials on human using a combination of anti-cancer drugs and anti-HER2 monoclonal antibody, trastuzumab, have been conducted to antagonize the proto-oncogene receptor HER2/neu; and have been achieving improved clinical response and overall survival of

breast-cancer patients (Lin et al., *Cancer Res.* 61:6345-9 (2001)). A tyrosine kinase inhibitor, STI-571, which selectively inactivates bcr-abl fusion proteins, has been developed to treat chronic myelogenous leukemias wherein constitutive activation of bcr-abl tyrosine kinase plays a crucial role in the transformation of leukocytes. Agents of these kinds are designed to 5 suppress oncogenic activity of specific gene products (Fujita et al., *Cancer Res.* 61:7722-6 (2001)). Therefore, gene products commonly up-regulated in cancerous cells may serve as potential targets for developing novel anti-cancer agents.

It has been demonstrated that CD8+ cytotoxic T lymphocytes (CTLs) recognize epitope peptides derived from tumor-associated antigens (TAAs) presented on MHC Class I molecule, 10 and lyse tumor cells. Since the discovery of MAGE family as the first example of TAAs, many other TAAs have been discovered using immunological approaches (Boon, *Int. J. Cancer* 54: 177-80 (1993); Boon and van der Bruggen, *J. Exp. Med.* 183: 725-9 (1996); van der Bruggen et al., *Science* 254: 1643-7 (1991); Brichard et al., *J. Exp. Med.* 178: 489-95 (1993); Kawakami et al., *J. Exp. Med.* 180: 347-52 (1994)). Some of the discovered TAAs are now in 15 the stage of clinical development as targets of immunotherapy. TAAs discovered so far include MAGE (van der Bruggen et al., *Science* 254: 1643-7 (1991)), gp100 (Kawakami et al., *J. Exp. Med.* 180: 347-52 (1994)), SART (Shichijo et al., *J. Exp. Med.* 187: 277-88 (1998)), and NY-ESO-1 (Chen et al., *Proc. Natl. Acad. Sci. USA* 94: 1914-8 (1997)). On the other hand, gene products which had been demonstrated to be specifically overexpressed in tumor cells, 20 have been shown to be recognized as targets inducing cellular immune responses. Such gene products include p53 (Umano et al., *Brit. J. Cancer* 84: 1052-7 (2001)), HER2/neu (Tanaka et al., *Brit. J. Cancer* 84: 94-9 (2001)), CEA (Nukaya et al., *Int. J. Cancer* 80: 92-7 (1999)), and so on.

In spite of significant progress in basic and clinical research concerning TAAs (Rosenbeg et 25 al., *Nature Med.* 4: 321-7 (1998); Mukherji et al., *Proc. Natl. Acad. Sci. USA* 92: 8078-82 (1995); Hu et al., *Cancer Res.* 56: 2479-83 (1996)), only limited number of candidate TAAs for the treatment of adenocarcinomas, including colorectal cancer, are available. TAAs abundantly expressed in cancer cells, and at the same time which expression is restricted to 30 cancer cells would be promising candidates as immunotherapeutic targets. Further, identification of new TAAs inducing potent and specific antitumor immune responses is expected to encourage clinical use of peptide vaccination strategy in various types of cancer (Boon and can der Bruggen, *J. Exp. Med.* 183: 725-9 (1996); van der Bruggen et al., *Science* 254: 1643-7 (1991); Brichard et al., *J. Exp. Med.* 178: 489-95 (1993); Kawakami et al., *J. Exp. Med.* 180: 347-52 (1994); Shichijo et al., *J. Exp. Med.* 187: 277-88 (1998); Chen et al., *Proc. 35 Natl. Acad. Sci. USA* 94: 1914-8 (1997); Harris, *J. Natl. Cancer Inst.* 88: 1442-5 (1996);

Butterfield et al., *Cancer Res.* 59: 3134-42 (1999); Vissers et al., *Cancer Res.* 59: 5554-9 (1999); van der Burg et al., *J Immunol* 156: 3308-14 (1996); Tanaka et al., *Cancer Res.* 57: 4465-8 (1997); Fujie et al., *Int. J. Cancer* 80: 169-72 (1999); Kikuchi et al., *Int. J. Cancer* 81: 459-66 (1999); Oiso et al., *Int. J. Cancer* 81: 387-94 (1999)).

5 It has been repeatedly reported that peptide-stimulated peripheral blood mononuclear cells (PBMCs) from certain healthy donors produce significant levels of IFN- γ in response to the peptide, but rarely exert cytotoxicity against tumor cells in an HLA-A24 or -A0201 restricted manner in ^{51}Cr -release assays (Kawano et al., *Cancer Res.* 60: 3550-8 (2000); Nishizaka et al., *Cancer Res.* 60: 4830-7 (2000); Tamura et al., *Jpn. J. Cancer Res.* 92: 762-7 (2001)). However,
10 both of HLA-A24 and HLA-A0201 are one of the popular HLA alleles in Japanese, as well as Caucasian (Date et al., *Tissue Antigens* 47: 93-101 (1996); Kondo et al., *J. Immunol.* 155: 4307-12 (1995); Kubo et al., *J. Immunol.* 152: 3913-24 (1994); Imanishi et al., *Proceeding of the eleventh International Histocompatibility Workshop and Conference Oxford University Press, Oxford*, 1065 (1992); Williams et al., *Tissue Antigen* 49: 129 (1997)). Thus, antigenic
15 peptides of cancers presented by these HLAs may be especially useful for the treatment of cancers among Japanese and Caucasian. Further, it is known that the induction of low-affinity CTL *in vitro* usually results from the use of peptide at a high concentration, generating a high level of specific peptide/MHC complexes on antigen presenting cells (APCs), which will effectively activate these CTL (Alexander-Miller et al., *Proc. Natl. Acad. Sci. USA* 93: 4102-7
20 (1996)).

Although advances have been made in the development of molecular-targeting drugs for cancer therapy, the ranges of tumor types that respond as well as the effectiveness of the treatments are still very limited. Hence, it is urgent to develop new anti-cancer agents that target molecules highly specific to malignant cells and are likely to cause minimal or no adverse reactions. To achieve the goal molecules whose physiological mechanisms are well defined need to be identified. A powerful strategy toward these ends would combine screening of up-regulated genes in cancer cells on the basis of genetic information obtained on cDNA microarrays with high-throughput screening of their effect on cell growth, by inducing loss-of-function phenotypes with RNAi systems (Kikuchi, T., Daigo, Y., Katagiri, T., Tsunoda, T., Okada, K., Kakiuchi, S., Zembutsu, H., Furukawa, Y., Kawamura, M., Kobayashi, K., Imai, K., Nakamura, Y. "Expression profiles of non-small cell lung cancers on cDNA microarrays: identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs", *Oncogene* 22: 2192-2205 (2003)).

SUMMARY OF THE INVENTION

The present invention features a method of diagnosing or determining a predisposition to non-small cell lung cancer (NSCLC) in a subject by determining an expression level of a non-small cell lung cancer-associated gene that is selected from the group of KIF11, GHSR1b, 5 and NTSR1 in a patient derived biological sample. An increase of the expression level of any of the genes compared to a normal control level of the genes indicates that the subject suffers from or is at risk of developing NSCLC.

A "normal control level" indicates an expression level of any of the genes detected in a normal, healthy individual or in a population of individuals known not to be suffering from 10 NSCLC. A control level is a single expression pattern derived from a single reference population or from a plurality of expression patterns. In contrast to a "normal control level", the "control level" is an expression level of the gene detected in an individual or a population of individuals whose background of the disease state is known (i.e., cancerous or non-cancerous). Thus, the control level may be determined base on the expression level of the gene in a normal, 15 healthy individual, in a population of individuals known not to be suffering from NSCLC, a patient of NSCLC or a population of the patients. The control level corresponding to the expression level of the gene in a patient of non-small cell lung cancer or a population of the patients is referred to as "NSCLC control level". Furthermore, the control level can be a database of expression patterns from previously tested cells.

20 An increase in the expression level of any one of the genes of KIF11, GHSR1b, and NTSR1 detected in a test biological sample compared to a normal control level indicates that the subject (from which the sample was obtained) suffers from NSCLC. Alternatively, the expression level of any one or all of the genes in a biological sample may be compared to an NSCLC control level of the same gene(s).

25 Gene expression is increased or decreased 10%, 25%, 50% or more compared to the control level. Alternatively, gene expression is increased or decreased 1, 2, 5 or more fold compared to the control level. Expression is determined by detecting hybridization, *e.g.*, on a chip or an array, of an NSCLC gene probe to a gene transcript of a patient-derived biological sample. The patient-derived biological sample may be any sample derived from a subject, *e.g.*, a patient 30 known to or suspected of having NSCLC. For example, the biological sample may be tissue containing sputum, blood, serum, plasma or lung cell.

The invention also provides a non-small cell lung cancer reference expression profile comprising a pattern of gene expression levels of two or more genes selected from the group of KIF11, GHSR1b, and NTSR1.

The invention also provides a kit comprising two or more detection reagents which detects the expression of one or more of genes selected from the group of KIF11, GHSR1b, and NTSR1 (e.g., via detecting mRNA and polypeptide). Also provided is an array of polynucleotides that binds to one or more of the genes selected from the group of KIF11, NTSR1, and GHSR1b.

5 GHSR1b, and NTSR1.

The invention further provides methods of identifying compounds that inhibit the expression level of an NSCLC-associated gene (KIF11, GHSR1b, or NTSR1) by contacting a test cell expressing an NSCLC-associated gene with a test compound and determining the expression level of the NSCLC-associated gene. The test cell may be an NSCLC cell. A decrease of the 10 expression level compared to a normal control level of the gene indicates that the test compound is an inhibitor of the expression or function of the NSCLC-associated gene. Therefore, if a compound suppresses the expression level of KIF11, GHSR1b, or NTSR1 compared to a normal control level, the compound is expected to reduce a symptom of NSCLC.

Alternatively, the present invention provides a method of screening for a compound for 15 treating or preventing NSCLC. The method includes contacting a polypeptide selected from the group of KIF11, GHSR1b and NTSR1 with a test compound, and selecting the test compound that binds to or suppresses the biological activity of the polypeptide. The invention further provides a method of screening for a compound for treating or preventing NSCLC, which includes the steps of contacting a test compound with a cell that expresses KIF11, 20 GHSR1b or NTSR1 protein or introduced with a vector comprising the transcriptional regulatory region of KIF11, GHSR1b or NTSR1 gene upstream of a reporter gene, and then selecting the test compound that reduces the expression level of the KIF11, GHSR1b or NTSR1 protein or protein encoded by the reporter gene. According to these screening methods, the test compound that suppresses the biological activity or the expression level compared to a 25 normal control level is expected to reduce a symptom of NSCLC. Furthermore, the present invention provides a method of screening for a compound for treating or preventing NSCLC wherein the binding between KIF11 and KOC1, or GHSR1b or NTSR1 and NMU is detected. Compounds that inhibit the binding between KIF11 and KOC1, or GHSR1b or NTSR1 and NMU are expected to reduce a symptom of NSCLC.

30 Methods for treating or preventing NSCLC and compositions to be used for such methods are also provided. Therapeutic methods include a method of treating or preventing NSCLC in a subject by administering to the subject a composition of an antisense, short interfering RNA (siRNA) or a ribozyme that reduce the expression of KIF11, GHSR1b or NTSR1 gene, or a composition comprising an antibody or fragment thereof that binds and suppresses the function

of a polypeptide encoded by the gene.

The invention also includes vaccines and vaccination methods. For example, a method of treating or preventing NSCLC in a subject is carried out by administering to the subject a vaccine containing a polypeptide encoded by KIF11, GHSR1b or NTSR1 gene, or an 5 immunologically active fragment of the polypeptide. An immunologically active fragment is a polypeptide that is shorter in length than the full-length naturally-occurring protein and which induces an immune response upon introduction into the body. For example, an immunologically active fragment includes a polypeptide of at least 8 residues in length that stimulates an immune cell such as a T cell or a B cell *in vivo*. Immune cell stimulation can be 10 measured by detecting cell proliferation, elaboration of cytokines (e.g., IL-2) or production of antibody.

Other therapeutic methods include those wherein a compound selected by the screening method of the present invention is administered.

Also included in the invention are double-stranded molecules that comprise a sense strand 15 and an antisense strand. The sense strand comprises a ribonucleotide sequence corresponding to a target sequence comprised within the mRNA of a KIF11, GHSR1b or NTSR1 gene, and the antisense strand is a complementary sequence to the sense strand. Such double-stranded molecules of the present invention can be used as siRNAs against KIF11, GHSR1b or NTSR1 gene. Furthermore, the present invention relates to vectors encoding the double-stranded 20 molecules of the present invention.

The present application also provides a composition for treating and/or preventing NSCLC using any of the antisense polynucleotides or siRNAs against KIF11, GHSR1b or NTSR1 gene, or an antibody that binds to a polypeptide encoded by KIF11, GHSR1b or NTSR1 gene. Other compositions include those that contain a compound selected by the screening method of 25 the present invention as an active ingredient.

It is to be understood that both the foregoing summary of the invention and the following detailed description are of a preferred embodiment, and not restrictive of the invention or other alternate embodiments of the invention.

30 **BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 shows a photograph depicting the result of SDS-PAGE identifying the KOC1-interacting protein. The protein complex including KOC1 was purified from LC319 cells that were transfected with bilateral-tagged (pCAGGS-n3FH vector: N-terminal; Flag, C-terminal;

HA) KOC1-expressing vector by immunoprecipitation (IP) (two step IP: 1st IP by anti-Flag agarose, followed by the 2nd IP by anti-HA antibody). As a control, mock- and other gene-construct-transfected LC319 cells were prepared. Immunoprecipitated products were stained by silver staining on the SDS-PAGE gel. A 120 kDa band was extracted and determined to be KIF11 by Mass spectrometric sequencing (in the figure, indicated with an arrow head).

5 Fig. 2 shows photographs confirming the relationship between KOC1 and KIF11. Fig. 2A depicts the result of co-immunoprecipitation of KOC1 and KIF11 confirming the interaction between KOC1 and KIF11. A549 cells were transiently co-transfected with 10 Flag-tagged KIF11 and myc-tagged KOC1, immunoprecipitated with anti-Flag M2 agarose, and subsequently immunoblotted with anti-myc antibody. In contrast, using the same combination of vectors and cells, the cells were immunoprecipitated with anti-myc agarose and immunoblotted with anti-Flag M2 antibody. A band corresponding to the immunoblotted protein was found only when both constructs were co-transfected. Fig. 15 2B depicts the result of immunocytochemical staining showing the co-localization of KOC1 and KIF11. COS-7 cells were transiently transfected with FLAG-tagged KIF11 and myc-tagged KOC1, and their co-localization was detected mainly in the cytoplasm using FITC-labeled anti-FLAG antibody and rhomamine-labeled anti-myc antibody.

20 Fig. 3 shows the result of semiquantitative RT-PCR demonstrating the expression of KOC1 and KIF11 in clinical samples of NSCLC, corresponding normal lung tissues, and NSCLC cell lines. Similar mRNA expression pattern was found between KIF11 and KOC1 in NSCLC.

25 Fig. 4 shows KIF11 expression in normal human tissues. Similar mRNA expression pattern was found between KIF11 and KOC1 in normal human tissues.

30 Fig. 5 shows the effect of siRNAs on cells. Fig. 5A depicts the inhibition on the growth of NSCLC cells by siRNAs against KIF11. The expression of KIF11 in response to specific siRNAs (si-KIF#1, #2, #3, #4, and #5) or control siRNAs (EGFP, LUC, SC) in A549 cells, was analyzed by semiquantitative RT-PCR. Fig. 5B depicts the viability of A549 cells in response to si-KIF#1, #2, #3, #4, #5, EGFP, LUC, or SC, evaluated by triplicate MTT assays.

Fig. 6 shows structural and functional characteristics of KIF11. Fig. 6A is a schematic illustration of KIF11. KIF11 is a member of the BimC family of kinesin related proteins whose members are characterized by a conserved, globular motor domain at the

N-terminus followed by a non-conserved, rod-like helical coiled-coil domain and a BimC box at the C-terminus. Fig. 6B depicts a schematic model mechanism of mRNA localization by KOC1 and KIF11.

Fig. 7 shows the result of semiquantitative RT-PCR analysis depicting the expression of NMU, 5 candidate receptors, and their known ligands detected in NSCLC cell lines.

Fig. 8 shows the relationship between NMU and GHSR1b/NTSR1. Fig. 8A depicts the result 10 of immunocytochemical staining using FITC-labeled anti-FLAG antibody showing the co-localization of NMU and GHSR1b/NTSR1 on the cell surface of COS-7 cells that were transiently transfected with FLAG-tagged GHSR1b or NTSR1. Fig. 8B depicts the interaction of NMU with GHSR1b/NTSR1. COS-7 cells were transiently 15 transfected with the same vectors, and binding of rhodamine-labeled NMU-25 to the cell surface was detected by flow cytometry. As negative controls for these assays, three ligand/cell combinations were prepared: 1) non-transfected COS-7 cells; 2) NMU-25-rhodamine vs non-transfected COS-7 cells; and 3) COS-7 cells transfected only with GHSR1b or NTSR1.

Fig. 9 shows GHSR1b expression in normal human tissues.

Fig. 10 shows the effect of siRNAs on cells. Fig. 10A depicts the inhibition on the growth of 20 NSCLC cells by siRNAs against GHSR1b and NTSR1. Expression of GHSR or NTSR1 in response to specific siRNAs (si-GHSR or si-NTSR1) or control siRNAs (EGFP, LUC, SCR) in A549 and LC319 cells were analyzed by semiquantitative RT-PCR. Fig. 10B depicts the result of triplicate MTT assays evaluating viability of A549 or LC319 cells in response to si-GHSR, NTSR1, EGFP, LUC, or SCR.

Fig. 11 is a schematic illustration of structural and functional characteristics of GHSR1b and 25 NTSR1.

25

DETAILED DESCRIPTION OF THE INVENTION

The words "a", "an" and "the" as used herein mean "at least one" unless otherwise 30 specifically indicated. The terms "protein" and "polypeptide" are used interchangeably. Furthermore, the terms "gene", "polynucleotide", and "nucleic acids" are used interchangeably unless otherwise specifically indicated.

To investigate the mechanisms of lung carcinogenesis and identify genes that might be useful as diagnostic markers or targets for development of new molecular therapies, genes specifically

up-regulated in non-small cell lung cancers (NSCLC) were searched by means of cDNA microarray. Through the analysis, a couple of candidate therapeutic target genes were identified. Two genes among them, KH domain containing protein over-expressed in cancer (KOC1) and neuromedin U (NMU) were abundantly expressed in clinical NSCLC samples as 5 well as NSCLC cell lines examined. However, their expressions were hardly detectable in corresponding non-cancerous lung tissue. The growth of NSCLC cells that over-expressed endogenous NMU was significantly inhibited by anti-NMU antibody. Furthermore, the treatment of NSCLC cells with siRNA against KOC1 or NMU suppressed the expression of the gene and resulted in growth inhibition of the NSCLC cells. Furthermore, KOC1 was 10 identified to bind to kinesin family member 11 (KIF11) of the cancer cells, whereas NMU bound to the neuropeptide G protein-coupled receptors (GPCRs), growth hormone secretagogue receptor 1b (GHSR1b) and neurotensin receptor 1 (NTSR1). Interestingly, GHSR1b, NTSR1, and KIF11 were all specifically over-expressed in NSCLC cells.

RNA binding protein KOC1 and microtubules motor protein KIF11 may be required for the 15 localization of some kinds of mRNA needed in embryogenesis and carcinogenesis (Fig. 6). As previously reported by the present inventors, treatment of NSCLC cells with specific siRNA to reduce expression of KOC1 resulted in growth suppression. In this study, KIF11 was demonstrated to associate with KOC1 in NSCLC cells and to be the likely target for the growth-promoting effect of KOC1 in lung tumors. The present inventors revealed that KOC1 20 not only co-localized with KIF11 in human normal tissues, NSCLCs, and cell lines, but also directly interacted with KIF11 in NSCLC cells *in vitro*, and that the treatment of NSCLC cells with siRNAs for KIF11 reduced its expression and led to growth suppression. The results suggest that KOC1-KIF11 signaling affects growth of NSCLC cells. By expression analysis, increased expression of KOC1 and KIF11 was detected in the majority of NSCLC samples, but 25 not in normal lung tissues. Since most of the clinical NSCLC samples used for the present analysis were at an early and operable stage, KOC1 and KIF11 might serve as a biomarker for diagnosing early-stage lung cancer, in combination with fiberoptic transbronchial biopsy (TBB) or sputum cytology.

Therefore, KOC1 and KIF11 are likely to be essential for an oncogenic pathway in NSCLCs. 30 The data reported here strongly imply the possibility of designing new anti-cancer drugs, specific for lung cancer, which target the KOC1-KIF11 pathway. They also suggest a potential for siRNAs themselves to interfere with this pathway, as a novel approach to treatment of chemotherapy-resistant, advanced lung cancers.

A significant increase in the sub-G1 fraction of NSCLC cells transfected with siRNA-NMU

suggested that blocking the autocrine NMU-signaling pathway could induce apoptosis. The present inventors also found other evidence supporting the significance of this pathway in carcinogenesis; e.g., addition of NMU into the medium promoted the growth of COS-7 cells in a dose-dependent manner, and addition of anti-NMU antibody into the culture medium 5 inhibited this NMU-enhanced cell growth, possibly by neutralizing NMU activity. Moreover, the growth of NSCLC cells that endogenously over-expressed NMU was significantly inhibited by anti-NMU antibody. The expression of NMU also resulted in significant promotion of COS-7 cell invasion in *in vitro* assays. These results strongly suggested that NMU is likely to be an important growth factor for NSCLC and might be associated with cancer cell invasion, 10 functioning in an autocrine manner, and that screening molecules targeting the NMU-receptor growth-promoting pathway should be a promising therapeutic approach for treating NSCLCs. By immunohistochemical analysis, increased expression of NMU protein was detected in the majority of NSCLC (S_{CC}, ADC, LCC, and BAC) and SCLC samples, but not in normal lung tissues. Since NMU is a secreted protein and most of the clinical NSCLC samples used for the 15 present analysis were at an early and operable stage, NMU might serve as a biomarker for diagnosis of early-stage lung cancer, in combination with fiberoptic transbronchial biopsy (TBB), sputum cytology, or blood tests.

Two receptors, NMU1R (FM3/GPR66) and NMU2R (FM4) are known to interact with NMU. The results presented here, however, indicated that these two known receptors were not the 20 targets for the autocrine NMU-signaling pathway in NSCLCs; instead, GHSR1b and NTSR1 proved to be the likely targets for the growth-promoting effect of NMU in lung tumors. The present inventors revealed that NMU-25 bound to these receptors on the cell surface, and that treatment of NSCLC cells with siRNAs for GHSR1b or NTSR1 reduced expression of the receptors and led to apoptosis. The results suggest that NMU affects growth of NSCLC cells 25 by acting through GHSR1b and/or NTSR1 (Fig. 11). GHSR is a known receptor of Ghrelin (GHRL), a recently identified 28-amino-acid peptide capable of stimulating release of pituitary growth hormone and appetite in humans (Lambert, P.D. et al. "Ciliary neurotrophic factor activates leptin-like pathways and reduces body fat, without cachexia or rebound weight gain, even in leptin-resistant obesity." Proc. Natl. Acad. Sci. 98: 4652-4657 (2001); Petersenn, S., 30 Rasch, A.C., Penshorn, M., Beil, F.U. & Schulte, H.M. "Genomic structure and transcriptional regulation of the human growth hormone secretagogue receptor." Endocrinology 142: 2649-2659 (2001); Kim K. et al. "Ghrelin and growth hormone (GH) secretagogue receptor (GHSR) mRNA expression in human pituitary adenomas." Clin. Endocrinol. 54: 705-860 (2001); Kojima, M. et al. "Ghrelin is a growth-hormone-releasing acylated peptide from 35 stomach." Nature 402: 656-660 (1999)). Of the two transcripts known to be receptors for

GHRL, GHSR1a and GHSR1b, over-expression of only GHSR1b was detected in NSCLC tissues and cell lines. Since GHRL was not expressed in the NSCLCs examined, GHSR1b was suspected to have a growth-promoting function in lung tumors through binding to NMU, but not to GHRL.

5 NTSR1 is one of three receptors of neuropeptide NTS, a brain and gastrointestinal peptide that fulfills many central and peripheral functions (Heasley, L.E. "Autocrine and paracrine signaling through neuropeptide receptors in human cancer." *Oncogene* 20: 1563-1569 (2001)). NTS modulates transmission of dopamine and secretion of pituitary hormones, and exerts hypothermic and analgesic effects in the brain while it functions as a peripheral hormone in the
10 digestive tract and cardiovascular system. Others have reported that NTS is produced and secreted in several human cancers, including small-cell lung cancers (SCLC) (Heasley, L.E. "Autocrine and paracrine signaling through neuropeptide receptors in human cancer." *Oncogene* 20: 1563-1569 (2001)). The expression of NTS was detected in four of the 15 NSCLC cell lines that were examined in the present invention (Fig. 7), but the expression
15 pattern of NTS was not necessarily concordant with that of NMU or NTSR1. Therefore NTS may, along with NMU, contribute to the growth of NSCLC through NTSR1 or other receptor(s) in a small subset of NSCLCs. In the present experiments the majority of the cancer cell lines and clinical NSCLCs that expressed NMU also expressed GHSR1b and/or NTSR1, indicating that these ligand-receptor interactions were likely to be involved in a pathway that is central to
20 the growth-promoting activity of NMU in NSCLCs.

Therefore, NMU and two newly revealed receptors for this molecule, GHSR1b and NTSR1, are likely to be essential for an autocrine growth-promoting pathway in NSCLCs. The data reported here strongly imply the possibility of designing new anti-cancer drugs, specific for lung cancer, that target the NMU-GHSR1b/NTSR1 pathway. They also suggest a potential for
25 siRNAs themselves to interfere with this pathway, as a novel approach to treatment of chemotherapy-resistant, advanced lung cancers.

These data strongly suggest that KOC1-KIF11 signaling pathway is frequently up-regulated in lung carcinogenesis, and that NMU is likely to be an important autocrine growth factor for NSCLC, acting through GHSR1b and NTSR1 receptor molecules. Thus, selective suppression
30 of components of these complexes may function to suppress the development and/or progression of lung carcinogenesis and targeting these pathways might provide promising therapeutic and diagnostic strategies for the treatment of lung-cancer patients.

Diagnosing non-small cell lung cancer (NSCLC)

By measuring the expression level of KIF11, GHSR1b or NTSR1 gene in a biological sample derived from a subject, the occurrence of NSCLC or a predisposition to develop NSCLC in the subject can be determined. The invention involves determining (e.g., measuring) the expression level of at least one, and up to all of KIF11, GHSR1b and NTSR1 gene in the biological sample.

According to the present invention, a gene transcript of NSCLC-associated gene, KIF11, GHSR1b or NTSR1, is detected for determining the expression level of the gene. The expression level of a gene can be detected by detecting the expression products of the gene, including both transcriptional and translational products, such as mRNA and proteins. Based on the sequence information provided by the GenBank™ database entries for the known sequences, KIF11, GHSR1b and NTSR1 genes can be detected and measured using techniques well known to one of ordinary skill in the art. The nucleotide sequences of the KIF11, GHSR1b and NTSR1 genes are described as SEQ ID NOs: 1, 3 and 5, respectively, and the amino acid sequences of the proteins encoded by the genes are described as SEQ ID NOs: 2, 4 and 6.

For example, sequences within the sequence database entries corresponding to KIF11, GHSR1b or NTSR1 gene can be used to construct probes for detecting their mRNAs by, e.g., Northern blot hybridization analysis. The hybridization of the probe to a gene transcript in a subject biological sample can be also carried out on a DNA array. The use of an array is preferred for detecting the expression level of a plurality of the NSC genes (KIF11, GHSR1b and NTSR1). As another example, the sequences can be used to construct primers for specifically amplifying KIF11, GHSR1b or NTSR1 gene in, e.g., amplification-based detection methods such as reverse-transcription based polymerase chain reaction (RT-PCR). Furthermore, the expression level of KIF11, GHSR1b or NTSR1 gene can be analyzed based on the quantity of the expressed proteins encoded by the gene. A method for determining the quantity of the expressed protein includes immunoassay methods. Alternatively, the expression level of KIF11, GHSR1b or NTSR1 gene can also be determined based on the biological activity of the expressed protein encoded by the gene. For example, a protein encoded by KIF11 gene is known to bind to KOC1, and thus the expression level of the gene can be detected by measuring the binding ability to KOC1 due to the expressed protein. Furthermore, KIF11 protein is known to have a cell proliferating activity. Therefore, the expression level of KIF11 gene can be determined using such cell proliferating activity as an index. On the other hand GHSR1b and NTSR1 proteins are known to bind to NMU, and also have a cell proliferating activity. Thus,

similarly to KIF11, the expression levels of GHSR1b and NTSR1 genes can be detected by measuring their binding ability to NMU or cell proliferating activity due to the expressed protein.

Any biological materials may be used as the biological sample for determining the expression level so long as any of the KIF11, GHSR1b and NTSR1 genes can be detected in the sample and 5 includes test cell populations (*i.e.*, subject derived tissue sample). Preferably, the biological sample comprises a lung cell (a cell obtained from the lung). Gene expression may also be measured in blood, serum or other bodily fluids such as sputum. Furthermore, the test sample may be cells purified from a tissue.

The subject diagnosed for NSCLC according to the method is preferably a mammal and 10 includes human, non-human primate, mouse, rat, dog, cat, horse and cow.

The expression level of one or more of KIF11, GHSR1b or NTSR1 gene in the biological sample is compared to the expression level(s) of the same genes in a reference sample. The reference sample includes one or more cells with known parameters, *i.e.*, cancerous or non-cancerous. The reference sample should be derived from a tissue type similar to that of 15 the test sample. Alternatively, the control expression level may be determined based on a database of molecular information derived from cells for which the assayed parameter or condition is known.

Whether or not a pattern of the gene expression levels in a biological sample indicates the presence of NSCLC depends upon the composition of the reference cell population. For 20 example, when the reference cell population is composed of non-cancerous cells, a similar gene expression level in the test biological sample to that of the reference indicates that the test biological sample is non-cancerous. On the other hand, when the reference cell population is composed of cancerous cells, a similar gene expression profile in the biological sample to that of the reference indicates that the test biological sample includes cancerous cells.

25 The test biological sample may be compared to multiple reference samples. Each of the multiple reference samples may differ in the known parameter. Thus, a test sample may be compared to a reference sample known to contain, *e.g.*, NSCLC cells, and at the same time to a second reference sample known to contain, *e.g.*, non-NSCLC cells (normal cells).

According to the invention, the expression of one or more of the NSCLC-associated genes, 30 KIF11, GHSR1b and NTSR1, is determined in the biological sample and compared to the normal control level of the same gene. The phrase "normal control level" refers to an expression profile of KIF11, GHSR1b or NTSR1 gene typically found in a biological sample derived from a population not suffering from NSCLC. The expression level of KIF11,

GHSR1b or NTSR1 gene in the biological samples from a control and test subjects may be determined at the same time or the normal control level may be determined by a statistical method based on the results obtained by analyzing the expression level of the gene in samples previously collected from a control group. An increase of the expression level of KIF11,

5 GHSR1b or NTSR1 gene in the biological sample derived from a patient derived tissue sample indicates that the subject is suffering from or is at risk of developing NSCLC.

An expression level of KIF11, GHSR1b or NTSR1 gene in a test biological sample can be considered altered when the expression level differs from that of the reference by more than 1.0, 1.5, 2.0, 5.0, 10.0 or more fold. Alternatively, an expression level of KIF11, GHSR1b or

10 NTSR1 gene in a test biological sample can be considered altered, when the expression level is increased or decreased to that of the reference at least 50%, 60%, 80%, 90% or more.

The difference in gene expression between the test sample and a reference sample may be normalized to a control, e.g., housekeeping gene. For example, a control polynucleotide includes those whose expression levels are known not to differ between the cancerous and

15 non-cancerous cells. The expression levels of the control polynucleotide in the test and reference samples can be used to normalize the expression levels detected for KIF11, GHSR1b or NTSR1 gene. The control genes to be used in the present invention include β -actin, glyceraldehyde 3-phosphate dehydrogenase and ribosomal protein P1.

The differentially expressed KIF11, GHSR1b or NTSR1 gene identified herein also allow for

20 monitoring the course of treatment of NSCLC. In this method, a test biological sample is provided from a subject undergoing treatment for NSCLC. If desired, multiple test biological samples are obtained from the subject at various time points before, during or after the treatment. The expression of one or more of KIF11, GHSR1b or NTSR1 gene in the sample is then determined and compared to a reference sample with a known state of NSCLC that has not

25 been exposed to the treatment.

If the reference sample contains no NSCLC cells, a similarity in the expression level of KIF11, GHSR1b or NTSR1 gene in the test biological sample and the reference sample indicates the efficaciousness of the treatment. However, a difference in the expression level of KIF11, GHSR1b or NTSR1 gene in the test and the reference samples indicates a less favorable

30 clinical outcome or prognosis.

The term "efficacious" refers that the treatment leads to a reduction in the expression of a pathologically up-regulated gene (including the present indicator genes, KIF11, GHSR1b and NTSR1), or a decrease in size, prevalence or metastatic potential of NSCLC in a subject.

When a treatment is applied prophylactically, "efficacious" means that the treatment retards or prevents occurrence of NSCLC or alleviates a clinical symptom of NSCLC. The assessment of NSCLC can be made using standard clinical protocols. Furthermore, the efficaciousness of a treatment is determined in association with any known method for diagnosing or treating

5 NSCLC. For example, NSCLC is diagnosed histopathologically or by identifying symptomatic anomalies such as chronic cough, hoarseness, coughing up blood, weight loss, loss of appetite, shortness of breath, wheezing, repeated bouts of bronchitis or pneumonia and chest pain.

Moreover, the present method for diagnosing NSCLC may also be applied for assessing the

10 prognosis of a patient with the cancer by comparing the expression level of KIF11, GHSR1b or NTSR1 gene in the patient-derived biological sample. Alternatively, the expression level of the gene(s) in the biological sample may be measured over a spectrum of disease stages to assess the prognosis of the patient.

An increase in the expression level of KIF11, GHSR1b or NTSR1 gene compared to a normal

15 control level indicates less favorable prognosis. A similarity in the expression level of KIF11, GHSR1b or NTSR1 gene compared to a normal control level indicates a more favorable prognosis of the patient. Preferably, the prognosis of a subject can be assessed by comparing the expression profile of KIF11, GHSR1b or NTSR1 gene.

20 *Expression profile*

The invention also provides an NSCLC reference expression profile comprising a pattern of gene expression levels of two or more of KIF11, GHSR1b and NTSR1 genes. The expression profile serves as a control for the diagnosis of NSCLC or predisposition for developing the disease, monitoring the course of treatment and assessing prognosis of a subject with the disease.

25

Kit

The invention also provides a kit comprising two or more detection reagents, *e.g.*, a nucleic acid that specifically binds to or identifies one or more of KIF11, GHSR1b and NTSR1 genes. Such nucleic acids specifically binding to or identifying one or more of KIF11, GHSR1b and

30 NTSR1 genes are exemplified by oligonucleotide sequences that are complementary to a portion of KIF11, GHSR1b or NTSR1 polynucleotides or antibodies which bind to polypeptides encoded by the KIF11, GHSR1b or NTSR1 gene. The reagents are packaged together in the form of a kit. The reagents, such as a nucleic acid or antibody (either bound to a solid matrix

or packaged separately with reagents for binding them to the matrix), a control reagent (positive and/or negative) and/or a means of detection of the nucleic acid or antibody are preferably packaged in separate containers. Instructions (e.g., written, tape, VCR, CD-ROM, etc.) for carrying out the assay may be included in the kit. The assay format of the kit may be Northern 5 hybridization or sandwich ELISA known in the art.

For example, a detection reagent is immobilized on a solid matrix such as a porous strip to form at least one detection site. The measurement or detection region of the porous strip may include a plurality of detection sites, each detection site containing a detection reagent. A test strip may also contain sites for negative and/or positive controls. Alternatively, control site(s) 10 is located on a separate strip from the test strip. Optionally, the different detection sites may contain different amounts of immobilized reagents, i.e., a higher amount in the first detection site and lesser amounts in subsequent sites. Upon the addition of a test biological sample, the number of sites displaying a detectable signal provides a quantitative indication of the amount of KIF11, GHSR1b or NTSR1 gene, or polypeptides encoded by the gene present in the sample. 15 The detection sites may be configured in any suitably detectable shape and are typically in the shape of a bar or dot spanning the width of a teststrip.

Alternatively, the kit contains a nucleic acid substrate array comprising two or more of the KIF11, GHSR1b and NTSR1 gene sequences. The expression of 2 or 3 of the genes represented by KIF11, GHSR1b and NTSR1 genes are identified by virtue of the level of 20 binding to an array test strip or chip. The substrate array can be on, e.g., a solid substrate, e.g., a "chip" as described in U.S. Patent No. 5,744,305.

Array and pluralities

The invention also includes a nucleic acid substrate array comprising one or more of the 25 KIF11, GHSR1b and NTSR1 genes. The nucleic acids on the array specifically correspond to one or more polynucleotide sequences represented by KIF11, GHSR1b and NTSR1 genes. The expression level of 2 or 3 of the KIF11, GHSR1b and NTSR1 genes is identified by detecting the binding of nucleic acid to the array.

The invention also includes an isolated plurality (i.e., a mixture of two or more nucleic acids) 30 of nucleic acids. The nucleic acids are in a liquid phase or a solid phase, e.g., immobilized on a solid support such as a nitrocellulose membrane. The plurality includes one or more of the polynucleotides represented by KIF11, GHSR1b and NTSR1 genes. According to a further embodiment of the present invention, the plurality includes 2 or 3 of the polynucleotides

represented by KIF11, GHSR1b and NTSR1 genes.

Chips

5 The DNA chip is a device that is convenient to compare the expression levels of a number of genes at the same time. DNA chip-based expression profiling can be carried out, for example, by the method as disclosed in "Microarray Biochip Technology" (Mark Schena, Eaton Publishing, 2000), etc.

10 A DNA chip comprises immobilized high-density probes to detect a number of genes. Thus, the expression levels of many genes can be estimated at the same time by a single-round analysis. Namely, the expression profile of a specimen can be determined with a DNA chip. The DNA chip-based method of the present invention comprises the following steps of:

- (1) synthesizing aRNAs or cDNAs corresponding to the marker genes;
- (2) hybridizing the aRNAs or cDNAs with probes for marker genes; and
- (3) detecting the aRNA or cDNA hybridizing with the probes and quantifying the amount of 15 mRNA thereof.

20 The aRNA refers to RNA transcribed from a template cDNA with RNA polymerase. An aRNA transcription kit for DNA chip-based expression profiling is commercially available. With such a kit, aRNA can be synthesized from T7 promoter-attached cDNA as a template using T7 RNA polymerase. On the other hand, by PCR using random primer, cDNA can be amplified using as a template a cDNA synthesized from mRNA.

25 Alternatively, the DNA chip comprises probes, which have been spotted thereon, to detect the marker genes of the present invention (KIF11, GHSR1b or NTSR1 gene). There is no limitation on the number of marker genes spotted on the DNA chip, and 1, 2 or all of the genes, KIF11, GHSR1b and NTSR1, may be used. Any other genes as well as the marker genes can be spotted on the DNA chip. For example, a probe for a gene whose expression level is hardly altered may be spotted on the DNA chip. Such a gene can be used to normalize assay results when the assay results are intended to be compared between multiple chips or between different assays.

30 A probe is designed for each marker gene selected, and spotted on a DNA chip. Such a probe may be, for example, an oligonucleotide comprising 5-50 nucleotide residues. A method for synthesizing such oligonucleotides on a DNA chip is known to those skilled in the

art. Longer DNAs can be synthesized by PCR or chemically. A method for spotting long DNA, which is synthesized by PCR or the like, onto a glass slide is also known to those skilled in the art. A DNA chip that is obtained by the method as described above can be used for diagnosing NSCLC according to the present invention.

5 The prepared DNA chip is contacted with aRNA, followed by the detection of hybridization between the probe and aRNA. The aRNA can be previously labeled with a fluorescent dye. A fluorescent dye such as Cy3(red) and Cy5 (green) can be used to label an aRNA. aRNAs from a subject and a control are labeled with different fluorescent dyes, respectively. The difference in the expression level between the two can be estimated based on a difference in the 10 signal intensity. The signal of fluorescent dye on the DNA chip can be detected by a scanner and analyzed using a special program. For example, the Suite from Affymetrix is a software package for DNA chip analysis.

Identifying compounds that inhibit NSCLC-associated gene expression

15 A compound that inhibits the expression or activity of a target NSCLC-associated gene (KIF11, GHSR1b or NTSR1 gene) is identified by contacting a test cell expressing the NSCLC-associated gene with a test compound and determining the expression level or activity of the NSCLC-associated gene. A decrease in expression compared to the normal control level indicates that the compound is an inhibitor of the NSCLC-associated gene. Such 20 compounds identified according to the method are useful for inhibiting NSCLC.

The test cell may be a population of cells and includes any cells as long as the cell expresses the target NSCLC-associated gene(s). For example, the test cell may be an immortalized cell line derived from an NSCLC cell. Alternatively, the test cell may be a cell transfected with any of the KIF11, GHSR1b and NTSR1 genes, or which has been transfected with the 25 regulatory sequence (e.g., promoter) of any of the genes that is operably linked to a reporter gene.

Screening compounds

Using KIF11, GHSR1b or NTSR1 gene, proteins encoded by the gene or transcriptional 30 regulatory region of the gene, compounds can be screened that alter the expression of the gene or biological activity of a polypeptide encoded by the gene. Such compounds are expected to serve as pharmaceuticals for treating or preventing NSCLC.

Therefore, the present invention provides a method of screening for a compound for treating or preventing NSCLC using the polypeptide of the present invention. An embodiment of this screening method comprises the steps of: (a) contacting a test compound with a polypeptide encoded by KIF11, GHSR1b or NTSR1 gene; (b) detecting the binding activity between the 5 polypeptide of the present invention and the test compound; and (c) selecting the compound that binds to the polypeptide.

The polypeptide to be used for the screening may be a recombinant polypeptide or a protein derived from the nature or a partial peptide thereof. The polypeptide to be contacted with a test compound can be, for example, a purified polypeptide, a soluble protein, a form bound to a 10 carrier or a fusion protein fused with other polypeptides.

As a method of screening for proteins that bind to KIF11, GHSR1b or NTSR1 polypeptide, many methods well known by a person skilled in the art can be used. Such a screening can be conducted by, for example, immunoprecipitation method, specifically, in the following manner. A gene encoding any of the KIF11, GHSR1b and NTSR1 polypeptides is expressed in animal 15 cells and so on by inserting the gene into an expression vector for foreign genes, such as pSV2neo, pcDNA I, pcDNA3.1, pCAGGS and pCD8. The promoter to be used for the expression may be any promoter that can be used commonly and include, for example, the SV40 early promoter (Rigby in Williamson (ed.), *Genetic Engineering*, vol. 3. Academic Press, London, 83-141 (1982)), the EF- α promoter (Kim et al., *Gene* 91: 217-23 (1990)), the CAG 20 promoter (Niwa et al., *Gene* 108: 193-200 (1991)), the RSV LTR promoter (Cullen, *Methods in Enzymology* 152: 684-704 (1987)) the SR α promoter (Takebe et al., *Mol Cell Biol* 8: 466 (1988)), the CMV immediate early promoter (Seed and Aruffo, *Proc Natl Acad Sci USA* 84: 3365-9 (1987)), the SV40 late promoter (Gheysen and Fiers, *J Mol Appl Genet* 1: 385-94 (1982)), the Adenovirus late promoter (Kaufman et al., *Mol Cell Biol* 9: 946 (1989)), the HSV 25 TK promoter and so on. The introduction of the gene into animal cells to express a foreign gene can be performed according to any methods, for example, the electroporation method (Chu et al., *Nucleic Acids Res* 15: 1311-26 (1987)), the calcium phosphate method (Chen and Okayama, *Mol Cell Biol* 7: 2745-52 (1987)), the DEAE dextran method (Lopata et al., *Nucleic Acids Res* 12: 5707-17 (1984); Sussman and Milman, *Mol Cell Biol* 4: 1642-3 (1985)), the 30 Lipofectin method (Derijard, *B Cell* 7: 1025-37 (1994); Lamb et al., *Nature Genetics* 5: 22-30 (1993); Rabindran et al., *Science* 259: 230-4 (1993)), and so on. The NSC polypeptide can also be expressed as a fusion protein comprising a recognition site (epitope) of a monoclonal antibody by introducing the epitope of the monoclonal antibody, whose specificity has been revealed, to the N- or C- terminus of the polypeptide. A commercially available 35 epitope-antibody system can be used (*Experimental Medicine* 13: 85-90 (1995)). Vectors

which can express a fusion protein with, for example, β -galactosidase, maltose binding protein, glutathione S-transferase, green fluorescence protein (GFP), and so on, by the use of its multiple cloning sites are commercially available.

5 A fusion protein prepared by introducing only small epitopes consisting of several to a dozen amino acids so as not to change the property of the original polypeptide by the fusion is also reported. Epitopes, such as polyhistidine (His-tag), influenza aggregate HA, human c-myc, FLAG, Vesicular stomatitis virus glycoprotein (VSV-GP), T7 gene 10 protein (T7-tag), human simple herpes virus glycoprotein (HSV-tag), E-tag (an epitope on monoclonal phage) and such, and monoclonal antibodies recognizing them can be used as the epitope-antibody system for 10 screening proteins binding to the KIF11, GHSR1b or NTSR1 polypeptide (Experimental Medicine 13: 85-90 (1995)).

In immunoprecipitation, an immune complex is formed by adding these antibodies to cell lysate prepared using an appropriate detergent. The immune complex consists of the KIF11, GHSR1b or NTSR1 polypeptide, a polypeptide comprising the binding ability with the 15 polypeptide, and an antibody. Immunoprecipitation can be also conducted using antibodies against the KIF11, GHSR1b or NTSR1 polypeptide, in addition to the use of antibodies against the above epitopes, which antibodies can be prepared according to conventional methods and may be in any form, such as monoclonal or polyclonal antibodies, and includes antiserum obtained by immunizing an animal such as a rabbit with the polypeptide, all classes of 20 polyclonal and monoclonal antibodies, as well as recombinant antibodies (e.g., humanized antibodies).

Herein, an antibody is defined as a protein that reacts with either the full length or a fragment of KIF11, GHSR1b or NTSR1 polypeptide. Specifically, antibodies against KIF11, GHSR1b or NTSR1 polypeptide can be prepared as follows. KIF11, GHSR1b or NTSR1 polypeptide 25 used as an antigen to obtain an antibody may be derived from any animal species, but preferably is derived from a mammal such as a human, mouse, or rat, more preferably from a human. According to the present invention, the polypeptide to be used as an immunization antigen may be a complete protein or a partial peptide of the KIF11, GHSR1b or NTSR1 polypeptide. A partial peptide may comprise, for example, the amino (N)-terminal or carboxy 30 (C)-terminal fragment of the KIF11, GHSR1b or NTSR1 polypeptide.

A gene encoding KIF11, GHSR1b or NTSR1 polypeptide, or its fragment may be inserted into a known expression vector, which is then used to transform a host cell. The desired protein or its fragment may be recovered from the outside or inside of host cells by any standard method, and may subsequently be used as an antigen. Alternatively, whole cells

expressing the KIF11, GHSR1b or NTSR1 polypeptide or their lysates, or a chemically synthesized polypeptide may be used as the antigen.

Any mammalian animal may be immunized with the antigen, but preferably the compatibility with parental cells used for cell fusion is taken into account. In general, animals of Rodentia, 5 Lagomorpha or Primates are used. Animals of Rodentia include, for example, mouse, rat and hamster. Animals of Lagomorpha include, for example, rabbit. Animals of Primates include, for example, a monkey of Catarrhini (old world monkey) such as *Macaca fascicularis*, rhesus monkey, sacred baboon and chimpanzees.

Methods for immunizing animals with antigens are known in the art. Intraperitoneal 10 injection or subcutaneous injection of antigens is a standard method for immunization of mammals. More specifically, antigens may be diluted and suspended in an appropriate amount of phosphate buffered saline (PBS), physiological saline, etc. If desired, the antigen suspension may be mixed with an appropriate amount of a standard adjuvant, such as Freund's complete adjuvant, made into emulsion, and then administered to mammalian animals. 15 Preferably, it is followed by several administrations of the antigen mixed with an appropriately amount of Freund's incomplete adjuvant every 4 to 21 days. An appropriate carrier may also be used for immunization. After immunization as above, the serum is examined by a standard method for an increase in the amount of desired antibodies.

20 Polyclonal antibodies against KIF11, GHSR1b or NTSR1 polypeptide may be prepared by collecting blood from the immunized mammal examined for the increase of desired antibodies in the serum, and by separating serum from the blood by any conventional method.

25 Polyclonal antibodies include serum containing the polyclonal antibodies, as well as the fraction containing the polyclonal antibodies may be isolated from the serum. Immunoglobulin G or M can be prepared from a fraction which recognizes only the KIF11, GHSR1b or NTSR1 polypeptide using, for example, an affinity column coupled with the polypeptide, and further purifying this fraction using protein A or protein G column.

To prepare monoclonal antibodies, immune cells are collected from the mammal immunized with the antigen and checked for the increased level of desired antibodies in the serum as 30 described above, and are subjected to cell fusion. The immune cells used for cell fusion are preferably obtained from spleen. Other preferred parental cells to be fused with the above immunocyte include, for example, myeloma cells of mammalians, and more preferably myeloma cells having an acquired property for the selection of fused cells by drugs.

The above immunocyte and myeloma cells can be fused according to known methods, for

example, the method of Milstein et al. (Galfre and Milstein, *Methods Enzymol* 73: 3-46 (1981)).

Resulting hybridomas obtained by the cell fusion may be selected by cultivating them in a standard selection medium, such as HAT medium (hypoxanthine, aminopterin, and thymidine containing medium). The cell culture is typically continued in the HAT medium for several days to several weeks, the time being sufficient to allow all the other cells, with the exception of the desired hybridoma (non-fused cells), to die. Then, the standard limiting dilution is performed to screen and clone a hybridoma cell producing the desired antibody.

In addition to the above method, in which a non-human animal is immunized with an antigen for preparing hybridoma, human lymphocytes such as those infected by EB virus may be immunized with KIF11, GHSR1b or NTSR1 polypeptide, cells expressing the polypeptide, or their lysates *in vitro*. Then, the immunized lymphocytes are fused with human-derived myeloma cells that are capable of indefinitely dividing, such as U266, to yield a hybridoma producing a desired human antibody that is able to bind to the KIF11, GHSR1b or NTSR1 polypeptide can be obtained (Unexamined Published Japanese Patent Application No. (JP-A) Sho 63-17688).

The obtained hybridomas are subsequently transplanted into the abdominal cavity of a mouse and the ascites are extracted. The obtained monoclonal antibodies can be purified by, for example, ammonium sulfate precipitation, a protein A or protein G column, DEAE ion exchange chromatography, or an affinity column to which any of the target proteins of the present invention (KIF11, GHSR1b and NTSR1 polypeptide) is coupled. The antibody can be used not only in the present screening method, but also for purification and detection of KIF11, GHSR1b or NTSR1 polypeptide, and serve also as candidates for agonists and antagonists of the polypeptide. In addition, this antibody can be applied to the antibody treatment for diseases related to the KIF11, GHSR1b or NTSR1 polypeptide including NSCLC as described *infra*.

Monoclonal antibodies thus obtained can be also recombinantly prepared using genetic engineering techniques (see, for example, Borrebaeck and Larrick, *Therapeutic Monoclonal Antibodies*, published in the United Kingdom by MacMillan Publishers LTD (1990)). For example, a DNA encoding an antibody may be cloned from an immune cell, such as a hybridoma or an immunized lymphocyte producing the antibody, inserted into an appropriate vector, and introduced into host cells to prepare a recombinant antibody. Such recombinant antibody can also be used for the present screening.

Furthermore, an antibody used in the screening and so on may be a fragment of an antibody or modified antibody, so long as it binds to one or more of KIF11, GHSR1b and NTSR1 polypeptides. For instance, the antibody fragment may be Fab, F(ab')₂, Fv, or single chain Fv (scFv), in which Fv fragments from H and L chains are ligated by an appropriate linker (Huston et al., Proc Natl Acad Sci USA 85: 5879-83 (1988)). More specifically, an antibody fragment may be generated by treating an antibody with an enzyme, such as papain or pepsin. Alternatively, a gene encoding the antibody fragment may be constructed, inserted into an expression vector, and expressed in an-appropriate host cell (see, for example, Co et al., J Immunol 152: 2968-76 (1994); Better and Horwitz, Methods Enzymol 178: 476-96 (1989); 5 Pluckthun and Skerra, Methods Enzymol 178: 497-515 (1989); Lamoyi, Methods Enzymol 10 121: 652-63 (1986); Rousseaux et al., Methods Enzymol 121: 663-9 (1986); Bird and Walker, Trends Biotechnol 9: 132-7 (1991)).

An antibody may be modified by conjugation with a variety of molecules, such as polyethylene glycol (PEG). Modified antibodies can be obtained through chemically 15 modification of an antibody. These modification methods are conventional in the field.

Alternatively, an antibody may be obtained as a chimeric antibody, between a variable region derived from nonhuman antibody and the constant region derived from human antibody, or as a humanized antibody, comprising the complementarity determining region (CDR) derived from nonhuman antibody, the frame work region (FR) derived from human antibody, and the 20 constant region. Such antibodies can be prepared using known technology.

Antibodies obtained as above may be purified to homogeneity. For example, the separation and purification of the antibody can be performed according to separation and purification methods used for general proteins. For example, the antibody may be separated and isolated by the appropriately selected and combined use of column chromatographies, such as affinity 25 chromatography, filter, ultrafiltration, salting-out, dialysis, SDS polyacrylamide gel electrophoresis, isoelectric focusing, and others (Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)), but are not limited thereto. A protein A column and protein G column can be used as the affinity column. Exemplary protein A columns to be used include, for example, Hyper D, POROS, and Sepharose F.F. 30 (Pharmacia).

Exemplary chromatography, with the exception of affinity includes, for example, ion-exchange chromatography, hydrophobic chromatography, gel filtration, reverse-phase chromatography, adsorption chromatography, and the like (Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Ed Daniel R. Marshak et al., Cold Spring

Harbor Laboratory Press (1996)). The chromatographic procedures can be carried out by liquid-phase chromatography, such as HPLC and FPLC.

An immune complex can be precipitated, for example with Protein A sepharose or Protein G sepharose when the antibody is a mouse IgG antibody. If the KIF11, GHSR1b or NTSR1 polypeptide is prepared as a fusion protein with an epitope, such as GST, an immune complex can be formed in the same manner as in the use of the antibody against the KIF11, GHSR1b or NTSR1 polypeptide, using a substance specifically binding to these epitopes, such as glutathione-Sepharose 4B.

5

10 Immunoprecipitation can be performed by following or according to, for example, the methods in the literature (Harlow and Lane, *Antibodies*, 511-52, Cold Spring Harbor Laboratory publications, New York (1988)).

15 SDS-PAGE is commonly used for analysis of immunoprecipitated proteins and the bound protein can be analyzed by the molecular weight of the protein using gels with an appropriate concentration. Since the protein bound to the KIF11, GHSR1b or NTSR1 polypeptide is difficult to detect by a common staining method, such as Coomassie staining or silver staining, the detection sensitivity for the protein can be improved by culturing cells in culture medium containing radioactive isotope, ³⁵S-methionine or ³⁵S-cystein, labeling proteins in the cells, and detecting the proteins. The target protein can be purified directly from the SDS-polyacrylamide gel and its sequence can be determined, when the molecular weight of the 20 protein has been revealed.

25 As a method for screening proteins binding to any of KIF11, GHSR1b and NTSR1 polypeptides using the polypeptide, for example, West-Western blotting analysis (Skolnik et al., Cell 65: 83-90 (1991)) can be used. Specifically, a protein binding to KIF11, GHSR1b or NTSR1 polypeptide can be obtained by preparing a cDNA library from cells, tissues, organs (for example, tissues such as lung cells) or cultured cells (particularly those derived from 30 NSCLC cells) expected to express a protein binding to the KIF11, GHSR1b or NTSR1 polypeptide using a phage vector (e.g., ZAP), expressing the protein on LB-agarose, fixing the protein expressed on a filter, reacting the purified and labeled KIF11, GHSR1b or NTSR1 polypeptide with the above filter, and detecting the plaques expressing proteins bound to the KIF11, GHSR1b or NTSR1 polypeptide according to the label. The KIF11, GHSR1b or NTSR1 polypeptide may be labeled by utilizing the binding between biotin and avidin, or by 35 utilizing an antibody that specifically binds to the KIF11, GHSR1b or NTSR1 polypeptide, or a peptide or polypeptide (for example, GST) that is fused to the KIF11, GHSR1b or NTSR1 polypeptide. Methods using radioisotope or fluorescence and such may be also used.

Alternatively, in another embodiment of the screening method of the present invention, a two-hybrid system utilizing cells may be used ("MATCHMAKER Two-Hybrid system", "Mammalian MATCHMAKER Two-Hybrid Assay Kit", "MATCHMAKER one-Hybrid system" (Clontech); "HybriZAP Two-Hybrid Vector System" (Stratagene); the references 5 "Dalton and Treisman, Cell 68: 597-612 (1992)", "Fields and Sternglanz, Trends Genet 10: 286-92 (1994)").

In the two-hybrid system, KIF11, GHSR1b or NTSR1 polypeptide is fused to the SRF-binding region or GAL4-binding region and expressed in yeast cells. A cDNA library is prepared from cells expected to express a protein binding to the KIF11, GHSR1b or NTSR1 10 polypeptide, such that the library, when expressed, is fused to the VP16 or GAL4 transcriptional activation region. The cDNA library is then introduced into the above yeast cells and the cDNA derived from the library is isolated from the positive clones detected (when a protein binding to the polypeptide of the invention is expressed in yeast cells, the binding of the two activates a reporter gene, making positive clones detectable). A protein encoded by the cDNA 15 can be prepared by introducing the cDNA isolated above to *E. coli* and expressing the protein.

As a reporter gene, for example, Ade2 gene, lacZ gene, CAT gene, luciferase gene and such can be used in addition to the HIS3 gene.

A compound binding to KIF11, GHSR1b or NTSR1 polypeptide can also be screened using affinity chromatography. For example, KIF11, GHSR1b or NTSR1 polypeptide may be 20 immobilized on a carrier of an affinity column, and a test compound, containing a protein capable of binding to KIF11, GHSR1b or NTSR1 polypeptide, is applied to the column. A test compound herein may be, for example, cell extracts, cell lysates, etc. After loading the test compound, the column is washed, and compounds bound to KIF11, GHSR1b or NTSR1 polypeptide can be prepared.

25 When the test compound is a protein, the amino acid sequence of the obtained protein is analyzed, an oligo DNA is synthesized based on the sequence, and cDNA libraries are screened using the oligo DNA as a probe to obtain a DNA encoding the protein.

A biosensor using the surface plasmon resonance phenomenon may be used as a mean for detecting or quantifying the bound compound in the present invention. When such a biosensor 30 is used, the interaction between KIF11, GHSR1b or NTSR1 polypeptide and a test compound can be observed real-time as a surface plasmon resonance signal, using only a minute amount of polypeptide and without labeling (for example, BIACore, Pharmacia). Therefore, it is possible to evaluate the binding between KIF11, GHSR1b or NTSR1 polypeptide and a test compound

using a biosensor such as BIACore.

The methods of screening for molecules that bind when an immobilized KIF11, GHSR1b or NTSR1 polypeptide is exposed to synthetic chemical compounds, or natural substance banks or a random phage peptide display library, and the methods of screening using high-throughput 5 based on combinatorial chemistry techniques (Wrighton et al., *Science* 273: 458-64 (1996); Verdine, *Nature* 384: 11-13 (1996); Hogan, *Nature* 384: 17-9 (1996)) to isolate not only proteins but chemical compounds that bind to KIF11, GHSR1b or NTSR1 protein (including agonist and antagonist) are well known to one skilled in the art.

Alternatively, the present invention provides a method of screening for a compound for 10 treating or preventing NSCLC using KIF11, GHSR1b or NTSR1 polypeptide comprising the steps as follows:

- (a) contacting a test compound with KIF11, GHSR1b or NTSR1 polypeptide;
- (b) detecting the biological activity of the KIF11, GHSR1b or NTSR1 polypeptide of step 15 (a); and
- (c) selecting a compound that suppresses the biological activity of the KIF11, GHSR1b or NTSR1 polypeptide in comparison with the biological activity detected in the absence of the test compound.

Since proteins encoded by any of the genes of KIF11, GHSR1b and NTSR1 have the activity 20 of promoting cell proliferation of NSCLC cells, a compound which inhibits this activity of one of these proteins can be screened using this activity as an index.

Any polypeptides can be used for screening so long as they comprise the biological activity of KIF11, GHSR1b or NTSR1 proteins. Such biological activity includes cell-proliferating activity and binding ability to other proteins of the proteins encoded by KIF11, GHSR1b or NTSR1 gene. For example, a human protein encoded by KIF11, GHSR1b or NTSR1 gene can 25 be used and polypeptides functionally equivalent to these proteins can also be used. Such polypeptides may be expressed endogenously or exogenously by cells.

The compound isolated by this screening is a candidate for antagonists of the KIF11, GHSR1b or NTSR1 polypeptide. The term "antagonist" refers to molecules that inhibit the function of KIF11, GHSR1b or NTSR1 polypeptide by binding thereto. Moreover, a 30 compound isolated by this screening is a candidate for compounds which inhibit the *in vivo* interaction of KIF11, GHSR1b or NTSR1 polypeptide with molecules (including DNAs and proteins).

When the biological activity to be detected in the present method is cell proliferation, it can be detected, for example, by preparing cells which express KIF11, GHSR1b or NTSR1 polypeptide, culturing the cells in the presence of a test compound, and determining the speed of cell proliferation, measuring the cell cycle and such, as well as by measuring the colony forming activity.

As discussed in detail above, by controlling the expression levels of KIF11, GHSR1b or NTSR1 gene, one can control the onset and progression of NSCLC. Thus, compounds that may be used in the treatment or prevention of NSCLC, can be identified through screenings that use the expression levels of one or more of KIF11, GHSR1b and NTSR1 genes as indices. In the context of the present invention, such screening may comprise, for example, the following steps:

- (a) contacting a test compound with a cell expressing one or more of KIF11, GHSR1b and NTSR1 genes; and
- (b) selecting a compound that reduces the expression level of one or more of the genes in comparison with the expression level detected in the absence of the test compound.

Cells expressing at least one of KIF11, GHSR1b and NTSR1 genes include, for example, cell lines established from NSCLC cells; such cells can be used for the above screening of the present invention (e.g., A549, NCI-H226, NCI-H522, LC319). The expression level can be estimated by methods well known to one skilled in the art. In the method of screening, a compound that reduces the expression level of at least one of the genes can be selected as candidate agents to be used for the treatment or prevention of NSCLC.

Alternatively, the screening method of the present invention may comprise the following steps:

- (a) contacting a test compound with a cell into which a vector comprising the transcriptional regulatory region of one or more of the marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the marker genes are selected from the group of KIF11, GHSR1b and NTSR1;
- (b) measuring the activity of said reporter gene; and
- (c) selecting a compound that reduces the expression level of said reporter gene as compared to a control.

Suitable reporter genes and host cells are well known in the art. The reporter construct required for the screening can be prepared using the transcriptional regulatory region of a

marker gene. When the transcriptional regulatory region of a marker gene has been known to those skilled in the art, a reporter construct can be prepared using the previous sequence information. When the transcriptional regulatory region of a marker gene remains unidentified, a nucleotide segment containing the transcriptional regulatory region can be 5 isolated from a genome library based on the nucleotide sequence information of the marker gene (e.g., based the 5' upstream sequence information).

In a further embodiment of the method of screening for a compound for treating or preventing NSCLC of the present invention, the method utilizes the binding ability of KIF11 to KOC1, or GHSR1b or NTSR1 to NMU.

10 As described above, the present inventors revealed that KOC1 not only co-localized with KIF11 in human normal tissues, NSCLCs, and cell lines, but also directly interacted with KIF11 in NSCLC cells *in vitro*, and that the treatment of NSCLC cells with siRNAs for KIF11 reduced its expression and led to growth suppression. The results suggest that KOC1-KIF11 signaling affects growth of NSCLC cells. Thus, it is expected that the inhibition of the binding between 15 KOC1 and KIF11 leads to the suppression of cell proliferation, and compounds inhibiting the binding serve as pharmaceuticals for treating or preventing NSCLCs. This screening method includes the steps of: (a) contacting a KIF11 polypeptide or functional equivalent thereof with KOC1 in the presence of a test compound; (b) detecting the binding between the polypeptide and KOC1; and (c) selecting the test compound that inhibits the binding between the 20 polypeptide and KOC1.

Furthermore, as described above, the present inventors revealed GHSR1b and NTSR1 as the likely targets for the growth-promoting effect of NMU in lung tumors. The present inventors revealed that NMU-25 bound to these receptors on the cell surface, and that treatment of NSCLC cells with siRNAs for GHSR1 or NTSR1 reduced expression of the receptors and led 25 to apoptosis. The results suggest that NMU affects growth of NSCLC cells by acting through GHSR1b and/or NTSR1 (Fig. 11). Thus, it is expected that the inhibition of binding between GHSR1b or NTSR1 and NMU leads to the suppression of cell proliferation, and compounds inhibiting the binding serve as pharmaceuticals for treating or preventing NSCLCs. This screening method includes the steps of: (a) contacting a GHSR1b or NTSR1 polypeptide or 30 functional equivalent thereof with NMU in the presence of a test compound; (b) detecting binding between the polypeptide and NMU; and (c) selecting the test compound that inhibits binding between the polypeptide and NMU.

KOC1 and KIF11 polypeptides, or GHSR1b or NTSR1 and NMU polypeptides to be used for the screening may be a recombinant polypeptide or a protein derived from the nature, or may

also be a partial peptide thereof so long as it retains the binding ability to each other. Such partial peptides retaining the binding ability are herein referred to as "functional equivalents". The KOC1 and KIF11 polypeptides, or GHSR1b or NTSR1 and NMU polypeptides to be used in the screening can be, for example, a purified polypeptide, a soluble protein, a form bound to 5 a carrier or a fusion protein fused with other polypeptides.

As a method of screening for compounds that inhibit binding between KOC1 and KIF11, or GHSR1b or NTSR1 and NMU, many methods well known by one skilled in the art can be used. Such a screening can be carried out as an *in vitro* assay system, for example, in a cellular system. More specifically, first, either KOC1 or KIF11, or GHSR1b or NTSR1, or NMU is 10 bound to a support, and the other protein is added together with a test compound thereto. Next, the mixture is incubated, washed and the other protein bound to the support is detected and/or measured.

Examples of supports that may be used for binding proteins include insoluble polysaccharides, such as agarose, cellulose and dextran; and synthetic resins, such as 15 polyacrylamide, polystyrene and silicon; preferably commercial available beads and plates (e.g., multi-well plates, biosensor chip, etc.) prepared from the above materials may be used. When using beads, they may be filled into a column. Alternatively, the use of magnetic beads of also known in the art, and enables to readily isolate proteins bound on the beads via magnetism.

The binding of a protein to a support may be conducted according to routine methods, such 20 as chemical bonding and physical adsorption. Alternatively, a protein may be bound to a support via antibodies specifically recognizing the protein. Moreover, binding of a protein to a support can be also conducted by means of avidin and biotin.

The binding between proteins is carried out in buffer, for example, but are not limited to, phosphate buffer and Tris buffer, as long as the buffer does not inhibit binding between the 25 proteins.

In the present invention, a biosensor using the surface plasmon resonance phenomenon may be used as a mean for detecting or quantifying the bound protein. When such a biosensor is used, the interaction between the proteins can be observed real-time as a surface plasmon resonance signal, using only a minute amount of polypeptide and without labeling (for example, 30 BIACore, Pharmacia). Therefore, it is possible to evaluate binding between the KOC1 and KIF11, or GHSR1b or NTSR1 and NMU using a biosensor such as BIACore.

Alternatively, either KOC1 or KIF11, or GHSR1b or NTSR1, or NMU may be labeled, and the label of the bound protein may be used to detect or measure the bound protein.

Specifically, after pre-labeling one of the proteins, the labeled protein is contacted with the other protein in the presence of a test compound, and then bound proteins are detected or measured according to the label after washing.

Labeling substances such as radioisotope (e.g., ^3H , ^{14}C , ^{32}P , ^{33}P , ^{35}S , ^{125}I , ^{131}I), enzymes (e.g., 5 alkaline phosphatase, horseradish peroxidase, β -galactosidase, β -glucosidase), fluorescent substances (e.g., fluorescein isothiosyanate (FITC), rhodamine) and biotin/avidin, may be used for the labeling of a protein in the present method. When the protein is labeled with radioisotope, the detection or measurement can be carried out by liquid scintillation. Alternatively, proteins labeled with enzymes can be detected or measured by adding a substrate 10 of the enzyme to detect the enzymatic change of the substrate, such as generation of color, with absorptiometer. Further, in case where a fluorescent substance is used as the label, the bound protein may be detected or measured using fluorophotometer.

Furthermore, binding of KOC1 and KIF11, or GHSR1b or NTSR1 and NMU can be also detected or measured using antibodies to the KOC1 and KIF11, or GHSR1b or NTSR1 and 15 NMU. For example, after contacting the KOC1 polypeptide immobilized on a support with a test compound and KIF11, the mixture is incubated and washed, and detection or measurement can be conducted using an antibody against KIF11. Alternatively, KIF11 may be immobilized on a support, and an antibody against KOC1 may be used as the antibody. When the combination of GHSR1b or NTSR1 and NMU is used, GHSR1b or NTSR1 polypeptide may be 20 immobilized on a support with a test compound and NMU, the mixture is incubated and washed, and detection or measurement can be conducted using an antibody against NMU. Alternatively, NMU may be immobilized on a support, and an antibody against GHSR1b or NTSR1 may be used as the antibody.

In case of using an antibody in the present screening, the antibody is preferably labeled with 25 one of the labeling substances mentioned above, and detected or measured based on the labeling substance. Alternatively, the antibody against KOC1 or KIF11, or GHSR1b or NTSR1, or NMU may be used as a primary antibody to be detected with a secondary antibody that is labeled with a labeling substance. Furthermore, the antibody bound to the protein in the screening of the present invention may be detected or measured using protein G or protein A 30 column.

Alternatively, in another embodiment of the screening method of the present invention, a two-hybrid system utilizing cells may be used ("MATCHMAKER Two-Hybrid system", "Mammalian MATCHMAKER Two-Hybrid Assay Kit", "MATCHMAKER one-Hybrid system" (Clontech); "HybriZAP Two-Hybrid Vector System" (Stratagene); the references

"Dalton and Treisman, Cell 68: 597-612 (1992)", "Fields and Sternglanz, Trends Genet 10: 286-92 (1994)".

In the two-hybrid system, for example, KOC1 polypeptide is fused to the SRF-binding region or GAL4-binding region and expressed in yeast cells. KIF11 polypeptide that binds to KOC1 polypeptide is fused to the VP16 or GAL4 transcriptional activation region and also expressed in the yeast cells in the existence of a test compound. Alternatively, KIF11 polypeptide may be fused to the SRF-binding region or GAL4-binding region, and KOC1 polypeptide to the VP16 or GAL4 transcriptional activation region. When the combination of GHSR1b or NTSR1 and NMU is used in the two-hybrid system, for example, GHSR1b or NTSR1 polypeptide is fused to the SRF-binding region or GAL4-binding region and expressed in yeast cells. NMU polypeptide that binds to GHSR1b or NTSR1 polypeptide is fused to the VP16 or GAL4 transcriptional activation region and also expressed in the yeast cells in the existence of a test compound. Alternatively, NMU polypeptide may be fused to the SRF-binding region or GAL4-binding region, and GHSR1b or NTSR1 polypeptide to the VP16 or GAL4 transcriptional activation region. When the test compound does not inhibit the binding between KOC1 and KIF11, or GHSR1b or NTSR1 and NMU, the binding of the two activates a reporter gene, making positive clones detectable.

As a reporter gene, for example, Ade2 gene, lacZ gene, CAT gene, luciferase gene and such can be used besides HIS3 gene.

Moreover, when the combination of GHSR1b or NTSR1 and NMU is used in the screening method, since GHSR1b and NTSR1 are polypeptides naturally expressed on the cell surface, in a preferable embodiment of the present screening method, the polypeptides are expressed on the surface of a living cell. When the polypeptides are expressed on the surface of a living cell, the binding between the polypeptide and NMU can be detected by methods detecting the autocrine and paracrine signaling leading to stimulation of tumor cell growth (Heasley, Oncogene 20: 1563-1569 (2001)). For example, the binding between GHSR1 or NTSR1 polypeptide and NMU can be detected by:

- (1) detecting the concentration of calcium or cAMP in the cell (e.g. FLIPR assay, Biochem. Biophys. Res. Commun. 276: 435-438, 2000; Nature 406: 70-74, 2000; J. Biol. Chem. 275: 21068-21074, 2000);
- (2) detecting the activation of the polypeptide;
- (3) detecting the interaction between the polypeptide and G-protein (e.g. FLIPR assay, Biochem. Biophys. Res. Commun. 276: 435-438, 2000; Nature 406: 70-74, 2000; J. Biol. Chem. 275: 21068-21074, 2000).

275:21068-21074, 2000, or binding assay with ^{125}I labeled peptide);

(4) detecting the activation of phospholipase C or its down stream pathway (Oncogene 20:1563-1569, 2001);

5 (5) detecting the activation of kinases of the protein kinase cascade, such as Raf, MEK, ERKs, and protein kinase D (PKD) (Oncogene 20:1563-1569, 2001);

(6) detecting the activation of a member of Src/Tec/Bmx-family of tyrosine kinases (Oncogene 20:1563-1569, 2001);

10 (7) detecting the activation of a member of the Ras and Rho family, regulation of a member of the JNK members of MAP families, or the reorganization of the actin cytoskeleton (Oncogene 20:1563-1569, 2001);

(8) detecting the activation of any signal complex mediated by the polypeptide activation;

15 (9) detecting the change in subcellular localization of the polypeptide including the ligand-induced internalization/endocytosis of the polypeptide (J. Cell Sci., 113: 2963-2975, 2000; J. Histochem. Cytochem. 48:1553-1563, 2000; Endocrinology October 23, 2003. as doi: 10.1210/en.2003-0974)

(10) detecting the activation of any transcription factor downstream of the polypeptides or the activation of their downstream gene; and

(11) detecting cell proliferation, transformation, or any other oncogenic phenotype of the cell.

Any test compound, for example, cell extracts, cell culture supernatant, products of fermenting microorganism, extracts from marine organism, plant extracts, purified or crude proteins, peptides, non-peptide compounds, synthetic micromolecular compounds and natural compounds can be used in the screening methods of the present invention. The test compound of the present invention can be also obtained using any of the numerous approaches in combinatorial library methods known in the art, including (1) biological libraries, (2) spatially addressable parallel solid phase or solution phase libraries, (3) synthetic library methods requiring deconvolution, (4) the "one-bead one-compound" library method and (5) synthetic library methods using affinity chromatography selection. The biological library methods using affinity chromatography selection is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, Anticancer Drug Des. 12: 145 (1997)). Examples of methods for the synthesis of molecular libraries can be found in the art (DeWitt et al., Proc. Natl. Acad. Sci.

USA 90: 6909 (1993); Erb et al., Proc. Natl. Acad. Sci. USA 91: 11422 (1994); Zuckermann et al., J. Med. Chem. 37: 2678 (1994); Cho et al., Science 261: 1303 (1993); Carell et al., Angew. Chem. Int. Ed. Engl. 33: 2059 (1994); Carell et al., Angew. Chem. Int. Ed. Engl. 33: 2061 (1994); Gallop et al., J. Med. Chem. 37: 1233 (1994)). Libraries of compounds may be 5 presented in solution (see Houghten, Bio/Techniques 13: 412 (1992)) or on beads (Lam, Nature 354: 82 (1991)), chips (Fodor, Nature 364: 555 (1993)), bacteria (US Pat. No. 5,223,409), spores (US Pat. No. 5,571,698; 5,403,484, and 5,223,409), plasmids (Cull et al., Proc. Natl. Acad. Sci. USA 89: 1865 (1992)) or phage (Scott and Smith, Science 249: 386 (1990); Delvin, Science 249: 404 (1990); Cwirla et al., Proc. Natl. Acad. Sci. USA 87: 6378 (1990); Felici, J. 10 Mol. Biol. 222: 301 (1991); US Pat. Application 2002103360). The test compound exposed to a cell or protein according to the screening methods of the present invention may be a single compound or a combination of compounds. When a combination of compounds are used in the screening methods of the invention, the compounds may be contacted sequentially or simultaneously.

15 A compound isolated by the screening methods of the present invention is a candidate for drugs which inhibit the activity of KIF11, GHSR1b or NTSR1 polypeptide, for treating or preventing diseases attributed to, for example, cell proliferative diseases, such as NSCLC. A compound in which a part of the structure of the compound obtained by the present screening methods of the present invention is converted by addition, deletion and/or replacement, is 20 included in the compounds obtained by the screening methods of the present invention. A compound effective in suppressing the expression of over-expressed genes, i.e., KIF11, GHSR1b or NTSR1 gene, is deemed to have a clinical benefit and can be further tested for its ability to prevent cancer cell growth in animal models or test subjects.

25 *Selecting a therapeutic agent for treating and/or preventing NSCLC that is appropriate for a particular individual*

Differences in the genetic makeup of individuals can result in differences in their relative abilities to metabolize various drugs. A compound that is metabolized in a subject to act as an anti-NSCLC agent can manifest itself by inducing a change in gene expression pattern in the 30 subject's cells from that characteristic of a cancerous state to a gene expression pattern characteristic of a non-cancerous state. Accordingly, the differentially expressed KIF11, GHSR1b and NTSR1 genes disclosed herein allow for selection of a putative therapeutic or prophylactic inhibitor of NSCLC specifically adequate for a subject by testing candidate compounds in a test cell (or test cell population) derived from the selected subject.

To identify an anti-NSCLC agent, that is appropriate for a specific subject, a test cell or test cell population derived from the subject is exposed to a therapeutic agent and the expression of one or more of the KIF11, GHSR1b and NTSR1 genes is determined.

The test cell is or the test cell population contains an NSCLC cell expressing an

5 NSCLC-associated gene. Preferably, the test cell is or the test cell population contains a lung cell. For example, the test cell or test cell population is incubated in the presence of a candidate agent and the pattern of gene expression of the test cell or cell population is measured and compared to one or more reference profiles, e.g., an NSCLC reference expression profile or an non-NSCLC reference expression profile.

10 A decrease in the expression of one or more of KIF11, GHSR1b and NTSR1 in a test cell or test cell population relative to a reference cell population containing NSCLC is indicative that the agent is therapeutic.

The test agent can be any compound or composition. For example, the test agent is an immunomodulatory agent.

15

Methods for treating or preventing NSCLC

The present invention provides a method for treating, alleviating or preventing NSCLC in a subject. Therapeutic compounds are administered prophylactically or therapeutically to subjects suffering from or at risk of (or susceptible to) developing NSCLC. Such subjects are

20 identified using standard clinical methods or by detecting an aberrant level of expression or activity of KIF11, GHSR1b or NTSR1 gene or polypeptide. Prophylactic administration occurs prior to the manifestation of overt clinical symptoms of disease, such that a disease or disorder is prevented or alternatively delayed in its progression.

The method includes decreasing the expression or function, or both, of one or more gene products of genes whose expression is aberrantly increased ("over-expressed gene"; KIF11, GHSR1b or NTSR1 gene) in an NSCLC cell relative to normal cells of the same tissue type from which the NSCLC cells are derived. The expression may be inhibited by any method known in the art. For example, a subject may be treated with an effective amount of a compound that decreases the amount of one or more of the KIF11, GHSR1b or NTSR1 gene in the subject. Administration of the compound can be systemic or local. Such therapeutic compounds include compounds that decrease the expression level of such gene that endogenously exists in the NSCLC cells (i.e., compounds that down-regulate the expression of the over-expressed gene(s), KIF11, GHSR1b and/or NTSR1 genes). The administration of

such therapeutic compounds counter the effects of aberrantly-over expressed gene(s) in the subjects NSCLC cells and are expected to improve the clinical condition of the subject. Such compounds can be obtained by the screening method of the present invention described above.

5 The compounds that modulate the activity of a protein encoded by KIF11, GHSR1b or NTSR1 gene that can be used for treating or preventing NSCLC of the present invention include besides proteins, naturally-occurring cognate ligand of these proteins, peptides, peptidomimetics and other small molecules.

10 Alternatively, the expression of these over-expressed gene(s) (KIF11, GHSR1b and/or NTSR1) can be inhibited by administering to the subject a nucleic acid that inhibits or 15 antagonizes the expression of the over-expressed gene(s). Antisense oligonucleotides, siRNAs or ribozymes which disrupt the expression of the over-expressed gene(s) can be used for inhibiting the expression of the over-expressed gene(s).

15 As noted above, antisense-oligonucleotides corresponding to any of the nucleotide sequence of KIF11, GHSR1b or NTSR1 gene can be used to reduce the expression level of the gene. 20 Antisense-oligonucleotides corresponding to KIF11, GHSR1b and NTSR1 genes that are up-regulated in NSCLC are useful for the treatment or prevention of NSCLC. Specifically, the antisense-oligonucleotides against the genes may act by binding to any of the corresponding polypeptides encoded by these genes, or mRNAs corresponding thereto, thereby inhibiting the transcription or translation of the genes, promoting the degradation of the mRNAs, and/or 25 inhibiting the expression of proteins encoded by the KIF11, GHSR1b and NTSR1 nucleotides, and finally inhibiting the function of the proteins. The term "antisense-oligonucleotides" as used herein encompasses both nucleotides that are entirely complementary to the target sequence and those having a mismatch of one or more nucleotides, so long as the antisense-oligonucleotides can specifically hybridize to the target sequence. For example, the 30 antisense-oligonucleotides of the present invention include polynucleotides that have a homology of at least 70% or higher, preferably at 80% or higher, more preferably 90% or higher, even more preferably 95% or higher over a span of at least 15 continuous nucleotides to any of the nucleotide sequence of KIF11, GHSR1b or NTSR1 gene. Algorithms known in the art can be used to determine the homology. Furthermore, derivatives or modified products of the antisense-oligonucleotides can also be used as antisense-oligonucleotides in the present invention. Examples of such modified products include lower alkyl phosphonate modifications such as methyl-phosphonate-type or ethyl-phosphonate-type, phosphorothioate modifications and phosphoroamidate modifications.

The antisense-oligonucleotides and derivatives thereof act on cells producing the proteins

encoded by KIF11, GHSR1b or NTSR1 gene by binding to the DNA or mRNA encoding the protein, inhibiting transcription or translation thereof, promoting the degradation of the mRNAs and inhibiting the expression of the protein, thereby resulting in the inhibition of the protein function.

5 An antisense-oligonucleotides and derivatives thereof can be made into an external preparation, such as a liniment or a poultice, by mixing with a suitable base material which is inactive against the derivative.

10 The antisense-oligonucleotides of the invention inhibit the expression of at least one protein encoded by any one of KIF11, GHSR1b and NTSR1 genes, and thus are useful for suppressing the biological activity of the protein.

15 The polynucleotides that inhibit one or more gene products of over-expressed genes also include small interfering RNAs (siRNA) comprising a combination of a sense strand nucleic acid and an antisense strand nucleic acid of the nucleotide sequence encoding an over-expressed protein encoded by KIF11, GHSR1b or NTSR1 gene. The term "siRNA" refers to a double stranded RNA molecule which prevents translation of a target mRNA. Standard techniques of introducing siRNA into the cell can be used in the treatment or prevention of the present invention, including those in which DNA is a template from which RNA is transcribed. The siRNA is constructed such that a single transcript has both the sense and complementary antisense sequences from the target gene, e.g., a hairpin.

20 The method is used to suppress gene expression of a cell with up-regulated expression of KIF11, GHSR1b or NTSR1 gene. Binding of the siRNA to KIF11, GHSR1b or NTSR1 gene transcript in the target cell results in a reduction of KIF11, GHSR1b or NTSR1 protein production by the cell. The length of the oligonucleotide is at least 10 nucleotides and may be as long as the naturally occurring transcript. Preferably, the oligonucleotide is 19-25
25 nucleotides in length. Most preferably, the oligonucleotide is less than 75, 50 or 25 nucleotides in length. Preferable siRNA of the present invention include the polynucleotides having the nucleotide sequence of SEQ ID NO: 32, 33, 34, 37, 39, or 40 as the target sequence, which all proved to be effective for suppressing cell viability of NSCLC cell lines. Specifically, a preferable siRNA used in the present invention has the general formula:

30 5'-[A]-[B]-[A']-3'

wherein [A] is a ribonucleotide sequence corresponding to a target sequence of KIF11, GHSR1b or NTSR1; [B] is a ribonucleotide sequence consisting of 3 to 23 nucleotides; and [A'] is a ribonucleotide sequence complementary to [A]. Herein, the phrase a "target sequence

of KIF11, GHSR1b or NTSR1 gene" refers to a sequence that, when introduced into NSCLC cell lines, is effective for suppressing cell viability. Preferred target sequence of KIF11, GHSR1b or NTSR1 gene includes nucleotide sequences comprising: SEQ ID NOS: 32, 33, 34, 37, 39, and 40. The complementary sequence [A'] and [A] hybridize to each other to form a double strand, and the whole siRNA molecule with the general formula 5'-[A]-[B]-[A']-3' forms a hairpin loop structure. As used herein, the term "complementary" refers to a Watson-Crick or Hoogsteen base pairing between nucleotide units of a polynucleotide, and hybridization or binding of nucleotide units indicates physical or chemical interaction between the units under appropriate conditions to form a stable duplex (double-stranded configuration) containing few or no mismatches. In a preferred embodiment, such duplexes contain no more than 1 mismatch for every 10 base pairs. Particularly preferred duplexes are fully complementary and contain no mismatch. The siRNA against the mRNA of KIF11, GHSR1b or NTSR1 gene to be used in the present invention contains a target sequence shorter than the whole mRNA of KIF11, GHSR1b or NTSR1 gene, and has a sequence of 500, 200, or 75 nucleotides as the whole length. Also included in the invention is a vector containing one or more of the nucleic acids described herein, and a cell containing the vectors. The isolated nucleic acids of the present invention are useful for siRNA against KIF11, GHSR1b or NTSR1 or DNA encoding the siRNA. When the nucleic acids are used for siRNA or coding DNA thereof, the sense strand is preferably longer than 19 nucleotides, and more preferably longer than 21 nucleotides.

Furthermore, the nucleotide sequence of siRNAs may be designed using a siRNA design computer program available from the Ambion website (http://www.ambion.com/techlib/misc/siRNA_finder.html). The nucleotide sequences for the siRNA are selected by the computer program based on the following protocol:

25 Selection of siRNA Target Sites:

1. Beginning with the AUG start codon of the transcript, scan downstream for AA dinucleotide sequences. Record the occurrence of each AA and the 3' adjacent 19 nucleotides as potential siRNA target sites. Tuschl, et al. recommend not to design siRNA against the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75 bases) as these may be richer in regulatory protein binding sites, and thus the complex of endonuclease and siRNAs that were designed against these regions may interfere with the binding of UTR-binding proteins and/or translation initiation complexes.
2. Compare the potential target sites to the human genome database and eliminate from consideration any target sequences with significant homology to other coding sequences.

The homology search can be performed using BLAST, which can be found on the NCBI server at: www.ncbi.nlm.nih.gov/BLAST/

3. Select qualifying target sequences for synthesis. On the website of Ambion, several preferable target sequences can be selected along the length of the gene for evaluation.
- 5 The siRNAs inhibit the expression of over-expressed KIF11, GHSR1b or NTSR1 protein and is thereby useful for suppressing the biological activity of the protein. Therefore, a composition comprising the siRNA is useful in treating or preventing non-small cell lung cancer.
- 10 The nucleic acids that inhibit one or more gene products of over-expressed genes KIF11, GHSR1b and NTSR1 also include ribozymes against the gene(s).

The ribozymes inhibit the expression of over-expressed KIF11, GHSR1b or NTSR1 protein and is thereby useful for suppressing the biological activity of the protein. Therefore, a composition comprising the ribozyme is useful in treating or preventing NSCLC.

Generally, ribozymes are classified into large ribozymes and small ribozymes. A large ribozyme is known as an enzyme that cleaves the phosphate ester bond of nucleic acids. After the reaction with the large ribozyme, the reacted site consists of a 5'-phosphate and 3'-hydroxyl group. The large ribozyme is further classified into (1) group I intron RNA catalyzing transesterification at the 5'-splice site by guanosine; (2) group II intron RNA catalyzing self-splicing through a two step reaction via lariat structure; and (3) RNA component of the ribonuclease P that cleaves the tRNA precursor at the 5' site through hydrolysis. On the other hand, small ribozymes have a smaller size (about 40 bp) compared to the large ribozymes and cleave RNAs to generate a 5'-hydroxyl group and a 2'-3' cyclic phosphate. Hammerhead type ribozymes (Koizumi et al., FEBS Lett. 228: 225 (1988)) and hairpin type ribozymes (Buzayan, Nature 323: 349 (1986); Kikuchi and Sasaki, Nucleic Acids Res. 19: 6751 (1992)) are included in the small ribozymes. Methods for designing and constructing ribozymes are known in the art (see Koizumi et al., FEBS Lett. 228: 225 (1988); Koizumi et al., Nucleic Acids Res. 17: 7059 (1989); Kikuchi and Sasaki, Nucleic Acids Res. 19: 6751 (1992)) and ribozymes inhibiting the expression of an over-expressed NSC protein can be constructed based on the sequence information of the nucleotide sequence encoding KIF11, GHSR1b or NTSR1 protein according to conventional methods for producing ribozymes.

The ribozymes inhibit the expression of over-expressed KIF11, GHSR1b or NTSR1 protein and is thereby useful for suppressing the biological activity of the protein. Therefore, a composition comprising the ribozyme is useful in treating or preventing NSCLC.

Alternatively, the function of one or more gene products of the over-expressed genes is inhibited by administering a compound that binds to or otherwise inhibits the function of the gene products. For example, the compound is an antibody which binds to the over-expressed gene product or gene products.

5 The present invention refers to the use of antibodies, particularly antibodies against a protein encoded by any of the up-regulated genes KIF11, GHSR1b or NTSR1, or a fragment of the antibody. As used herein, the term "antibody" refers to an immunoglobulin molecule having a specific structure that interacts (binds) specifically with a molecule comprising the antigen used for synthesizing the antibody (i.e., the up-regulated gene product) or with an antigen closely related to it. An antibody that binds to the over-expressed KIF11, GHSR1b or NTSR1 nucleotide may be in any form, such as monoclonal or polyclonal antibodies, and includes antiserum obtained by immunizing an animal such as a rabbit with the polypeptide, all classes of polyclonal and monoclonal antibodies, human antibodies and humanized antibodies produced by genetic recombination. Furthermore, the antibody used in the method of treating or preventing NSCLC of the present invention may be a fragment of an antibody or a modified antibody, so long as it binds to one or more of the proteins encoded by the marker genes (KIF11, GHSR1b or NTSR1 gene). The antibodies and antibody fragments used in the present method of treating or preventing NSCLC may be modified, and include chemically modified and chimeric antibodies. Such antibodies and antibody fragments can be obtained according to the above-mentioned methods, *supra*.

10

15

20

When the obtained antibody is to be administered to the human body (antibody treatment), a human antibody or a humanized antibody is preferable for reducing immunogenicity.

25 For example, transgenic animals having a repertory of human antibody genes may be immunized with an antigen such as KIF11, GHSR1b or NTSR1 polypeptide, cells expressing the polypeptide, or their lysates. Antibody producing cells are then collected from the animals and fused with myeloma cells to obtain hybridoma, from which human antibodies against the polypeptide can be prepared (see WO92-03918, WO93-2227, WO94-02602, WO94-25585, WO96-33735, and WO96-34096).

30 Alternatively, an immune cell, such as an immunized lymphocyte, producing antibodies may be immortalized by an oncogene and used for preparing monoclonal antibodies.

The present invention provides a method for treating or preventing NSCLC, using an antibody against an over-expressed KIF11, GHSR1b or NTSR1 polypeptide. According to the method, a pharmaceutically effective amount of an antibody against KIF11, GHSR1b or

NTSR1 polypeptide is administered. An antibody against an over-expressed KIF11, GHSR1b or NTSR1 polypeptide is administered at a dosage sufficient to reduce the activity of KIF11, GHSR1b or NTSR1 protein. Alternatively, an antibody binding to a cell surface marker specific for tumor cells can be used as a tool for drug delivery. Thus, for example, an antibody against an over-expressed KIF11, GHSR1b or NTSR1 polypeptide conjugated with a cytotoxic agent may be administered at a dosage sufficient to injure tumor cells.

The present invention also relates to a method of treating or preventing NSCLC in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a nucleic acid selected from the group consisting of KIF11, GHSR1b and NTSR1 genes or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide or the fragment thereof. Administration of the polypeptide induces an anti-tumor immunity in a subject. Thus, the present invention further provides a method for inducing anti tumor immunity. The polypeptide or the immunologically active fragments thereof are useful as vaccines against NSCLC. In some cases the proteins or fragments thereof may be administered in a form bound to the T cell receptor (TCR) or presented on an antigen presenting cell (APC), such as macrophage, dendritic cell (DC) or B-cells. Due to the strong antigen presenting ability of DC, the use of DC is most preferable among the APCs.

In the present invention, the phrase "vaccine against NSCLC" refers to a substance that has the function to induce anti-tumor immunity or immunity to suppress NSCLC upon inoculation into animals. In general, anti-tumor immunity includes immune responses such as follows:

- induction of cytotoxic lymphocytes against tumors,
- induction of antibodies that recognize tumors, and
- induction of anti-tumor cytokine production.

Therefore, when a certain protein induces any one of these immune responses upon inoculation into an animal, the protein is decided to have anti-tumor immunity inducing effect. The induction of the anti-tumor immunity by a protein can be detected by observing *in vivo* or *in vitro* the response of the immune system in the host against the protein.

For example, a method for detecting the induction of cytotoxic T lymphocytes is well known. A foreign substance that enters the living body is presented to T cells and B cells by the action of antigen presenting cells (APCs). T cells that respond to the antigen presented by APC in antigen specific manner differentiate into cytotoxic T cells (or cytotoxic T lymphocytes; CTLs) due to stimulation by the antigen, and then proliferate (this is referred to as activation of T cells).

Therefore, CTL induction by a certain peptide can be evaluated by presenting the peptide to T cell by APC, and detecting the induction of CTL. Furthermore, APC has the effect of activating CD4+ T cells, CD8+ T cells, macrophages, eosinophils and NK cells. Since CD4+ T cells are also important in anti-tumor immunity, the anti-tumor immunity inducing action of the peptide can be evaluated using the activation effect of these cells as indicators.

5 A method for evaluating the inducing action of CTL using dendritic cells (DCs) as APC is well known in the art. DC is a representative APC having the strongest CTL inducing action among APCs. In this method, the test polypeptide is initially contacted with DC and then this DC is contacted with T cells. Detection of T cells having cytotoxic effects against the cells of interest after the contact with DC shows that the test polypeptide has an activity of inducing the cytotoxic T cells. Activity of CTL against tumors can be detected, for example, using the lysis of ⁵¹Cr-labeled tumor cells as the indicator. Alternatively, the method of evaluating the degree of tumor cell damage using ³H-thymidine uptake activity or LDH (lactose dehydrogenase)-release as the indicator is also well known.

10 15 Apart from DC, peripheral blood mononuclear cells (PBMCs) may also be used as the APC. The induction of CTL is reported to be enhanced by culturing PBMC in the presence of GM-CSF and IL-4. Similarly, CTL is shown to be induced by culturing PBMC in the presence of keyhole limpet hemocyanin (KLH) and IL-7.

20 25 The test polypeptides confirmed to possess CTL inducing activity by these methods are polypeptides having DC activation effect and subsequent CTL inducing activity. Therefore, polypeptides that induce CTL against tumor cells are useful as vaccines against NSCLC. Furthermore, APC that acquired the ability to induce CTL against NSCLC by contacting with the polypeptides are useful as vaccines against NSCLC. Furthermore, CTL that acquired cytotoxicity due to presentation of the polypeptide antigens by APC can be also used as vaccines against NSCLC. Such therapeutic methods for NSCLC using anti-tumor immunity due to APC and CTL are referred to as cellular immunotherapy.

30 Generally, when using a polypeptide for cellular immunotherapy, efficiency of the CTL-induction is known to increase by combining a plurality of polypeptides having different structures and contacting them with DC. Therefore, when stimulating DC with protein fragments, it is advantageous to use a mixture of multiple types of fragments.

35 Alternatively, the induction of anti-tumor immunity by a polypeptide can be confirmed by observing the induction of antibody production against tumors. For example, when antibodies against a polypeptide are induced in a laboratory animal immunized with the polypeptide, and

when growth, proliferation or metastasis of tumor cells is suppressed by those antibodies, the polypeptide can be determined to have an ability to induce anti-tumor immunity.

Anti-tumor immunity is induced by administering the vaccine of this invention, and the induction of anti-tumor immunity enables treatment and prevention of NSCLC. Therapy

5 against or prevention of the onset of NSCLC includes any of the steps, such as inhibition of the growth of NSCLC cells, involution of NSCLC cells and suppression of occurrence of NSCLC cells. Decrease in mortality of individuals having NSCLC, decrease of marker genes (in addition to KIF11, GHSR1b and/or NTSR1 genes) in the blood, alleviation of detectable symptoms accompanying NSCLC and such are also included in the therapy or prevention of

10 NSCLC. Such therapeutic and preventive effects are preferably statistically significant. For example, in observation, at a significance level of 5% or less, wherein the therapeutic or preventive effect of a vaccine against NSCLC is compared to a control without vaccine administration. For example, Student's t-test, the Mann-Whitney U-test or ANOVA may be used for statistical analysis.

15 The above-mentioned protein having immunological activity, or a polynucleotide or vector encoding the protein may be combined with an adjuvant. An adjuvant refers to a compound that enhances the immune response against the protein when administered together (or successively) with the protein having immunological activity. Examples of adjuvants include cholera toxin, salmonella toxin, alum and such, but are not limited thereto. Furthermore, the

20 vaccine of this invention may be combined appropriately with a pharmaceutically acceptable carrier. Examples of such carriers are sterilized water, physiological saline, phosphate buffer, culture fluid and such. Furthermore, the vaccine may contain as necessary, stabilizers, suspensions, preservatives, surfactants and such. The vaccine is administered systemically or locally. Vaccine administration may be performed by single administration or boosted by

25 multiple administrations.

When using APC or CTL as the vaccine of this invention, NSCLC can be treated or prevented, for example, by the *ex vivo* method. More specifically, PBMCs of the subject receiving treatment or prevention are collected, the cells are contacted with the polypeptide *ex vivo*, and following the induction of APC or CTL, the cells may be administered to the subject.

30 APC can be also induced by introducing a vector encoding the polypeptide into PBMCs *ex vivo*. APC or CTL induced *in vitro* can be cloned prior to administration. By cloning and growing cells having high activity of damaging target cells, cellular immunotherapy can be performed more effectively. Furthermore, APC and CTL isolated in this manner may be used for cellular immunotherapy not only against individuals from whom the cells are derived, but also against

similar types of diseases in other individuals.

Moreover, the present invention provides a method for treating or preventing NSCLC in a subject, wherein a compound obtained according to any of the above-described screening methods is administered to the subject. Any compound that are obtained according to any of the screening methods of the present invention can be administered to the subject so long as it decreases the expression or function, or both, of one or more gene products of KIF11, GHSR1b and NTSR1 genes.

siRNA and vectors encoding them

10 Transfection of vectors expressing siRNA for KIF11, GHSR1b or NTSR1 leads to growth inhibition of NSCLC cells. Thus, it is another aspect of the present invention to provide a double-stranded molecule comprising a sense-strand and antisense-strand which molecule functions as an siRNA for KIF11, GHSR1b or NTSR1, and a vector encoding the double-stranded molecule.

15 The double-stranded molecule of the present invention comprises a sense strand and an antisense strand, wherein the sense strand comprises a ribonucleotide sequence corresponding to a KIF11, GHSR1b, or NTSR1 target sequence, and wherein the antisense strand comprises a ribonucleotide sequence which is complementary to said sense strand, wherein said sense strand and said antisense strand hybridize to each other to form said double-stranded molecule, and

20 wherein said double-stranded molecule, when introduced into a cell expressing a KIF11, GHSR1b, or NTSR1 gene, inhibits expression of said gene.

The double-stranded molecule of the present invention may be a polynucleotide derived from its original environment (i.e., when it is a naturally occurring molecule, the natural environment), physically or chemically altered from its natural state, or chemically synthesized.

25 According to the present invention, such double-stranded molecules include those composed of DNA, RNA, and derivatives thereof. A DNA is suitably composed of bases such as A, T, C and G, and T is replaced by U in an RNA.

As described above, the term "complementary" refers to a Watson-Crick or Hoogsteen base pairing between nucleotide units of a polynucleotide, and hybridization or binding of nucleotide units indicates physical or chemical interaction between the units under appropriate conditions to form a stable duplex (double-stranded configuration) containing few or no mismatches. In a preferred embodiment, such duplexes contain no more than 1 mismatch for every 10 base

pairs. Particularly preferred duplexes are fully complementary and contain no mismatch.

The double-stranded molecule of the present invention contains a ribonucleotide sequence corresponding to a KIF11, GHSR1b, or NTSR1 target sequence shorter than the whole mRNA of KIF11, GHSR1b or NTSR1 gene. Herein, the phrase a "target sequence of KIF11, GHSR1b or NTSR1 gene" refers to a sequence that, when introduced into NSCLC cell lines, is effective for suppressing cell viability. Specifically, the target sequence comprises at least about 10, or suitably about 19 to about 25 contiguous nucleotides from the nucleotide sequences selected from the group of SEQ ID NOS: 1, 3 and 5. That is, the sense strand of the present double-stranded molecule consists of at least about 10 nucleotides, suitably is longer than 19 nucleotides, and more preferably longer than 21 nucleotides. Preferred target sequences include the sequences of SEQ ID NOS: 32, 33, 34, 37, 39, and 40. The present double-stranded molecule including the sense strand and the antisense strand is an oligonucleotide shorter than about 100, preferably 75, more preferably 50 and most preferably 25 nucleotides in length. A suitable double-stranded molecule of the present invention is an oligonucleotide of a length of about 19 to about 25 nucleotides. Furthermore, in order to enhance the inhibition activity of the siRNA, nucleotide "u" can be added to 3'end of the antisense strand of the target sequence. The number of "u"s to be added is at least 2, generally 2 to 10, preferably 2 to 5. The added "u"s form single strand at the 3'end of the antisense strand of the siRNA.

Furthermore, the double-stranded molecule of the present invention may be a single ribonucleotide transcript comprising the sense strand and the antisense strand linked via a single-stranded ribonucleotide sequence. Namely, the present double-stranded molecule may have the general formula:

5'-[A]-[B]-[A']-3'

wherein [A] is a ribonucleotide sequence corresponding to a target sequence of KIF11, GHSR1b or NTSR1;

[B] is a ribonucleotide sequence (loop sequence) consisting of 3 to 23 nucleotides; and

[A'] is a ribonucleotide sequence complementary to [A]. The complementary sequence [A'] and [A] hybridize to each other to form a double strand, and the whole siRNA molecule with the general formula 5'-[A]-[B]-[A']-3' forms a hairpin loop structure.

The region [A] hybridizes to [A'], and then a loop consisting of region [B] is formed. The loop sequence can be selected from those described in

http://www.ambion.com/techlib/tb/tb_506.html, or those described in Jacque, J.-M., Triques, K., and Stevenson, M. "Modulation of HIV-1 replication by RNA interference." *Nature* 418: 435-438 (2002). Additional examples of the loop sequence that can be included in the present double-stranded molecules include:

5 CCC, CCACC or CCACACC: Jacque, J. M., Triques, K., and Stevenson, M. "Modulation of HIV-1 replication by RNA interference." *Nature*, Vol. 418: 435-438 (2002);
UUCG: Lee, N.S., Dohjima, T., Bauer, G., Li, H., Li, M.-J., Ehsani, A., Salvaterra, P., and Rossi, J. "Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells." *Nature Biotechnology* 20:500-505 (2002); Fruscoloni, P., Zamboni, M., and

10 Tocchini-Valentini, G. P. "Exonucleolytic degradation of double-stranded RNA by an activity in *Xenopus laevis* germinal vesicles." *Proc. Natl. Acad. Sci. USA* 100(4): 1639-1644 (2003); and
UUCAAGAGA: Dykxhoorn, D. M., Novina, C. D., and Sharp, P. A. "Killing the messenger: Short RNAs that silence gene expression." *Nature Reviews Molecular Cell Biology* 4: 457-467 (2002).

15

Preferable siRNAs having hairpin loop structure of the present invention are shown below. In the following structure, the loop sequence can be selected from the group consisting of: CCC, UUCG, CCACC, CCACACC, and UUCAAGAGA. Among these sequences, the most preferable loop sequence is UUCAAGAGA (corresponding to "ttcaagaga" in a DNA):

20 guuaguguacgaacuggag-[B]-cuccaguuucguacacuaac (for the target sequence of SEQ ID NO:32);
gugucucugu uggagaacu-[B]-agaucuccaacagagacac (for the target sequence of SEQ ID NO:33);
gaaggcaguu gaccaacac-[B]-guguugguacacugccuuc (for the target sequence of SEQ ID NO:34);
ccucuaccug uccagcaug-[B]-caugcuggacagguagagg (for the target sequence of SEQ ID NO:37);
guucaucagc gccaucugg-[B]-ccagauggcgcugaugaac (for the target sequence of SEQ ID NO:39);
25 and
ggucgucaua caggucaac-[B]-guugaccuguaugacgacc (for the target sequence of SEQ ID NO:40).

The present invention further provides a vector encoding the double-stranded molecule of the present invention. The vector encodes a transcript having a secondary structure and which comprises the sense strand and the antisense strand, and suitably comprises a single-stranded ribonucleotide sequence linking said sense strand and said antisense strand. The vector

5 preferably comprises a regulatory sequence adjacent to the region encoding the present double-stranded molecule that directs the expression of the molecule in an adequate cell. For example, the double-stranded molecules of the present invention are intracellularly transcribed by cloning their coding sequence into a vector containing, e.g., a RNA pol III transcription unit from the small nuclear RNA (snRNA) U6 or the human H1 RNA promoter.

10 Alternatively, the present vectors are produced, for example, by cloning the target sequence into an expression vector so the objective sequence is operatively-linked to a regulatory sequence of the vector in a manner to allow expression thereof (transcription of the DNA molecule) (Lee, N.S., Dohjima, T., Bauer, G., Li, H., Li, M.-J., Ehsani, A., Salvaterra, P., and Rossi, J. "Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells." *Nature Biotechnology* 20: 500-505 (2002)). For example, the transcription of an RNA molecule having an antisense sequence to the target sequence is driven by a first promoter (e.g., a promoter sequence linked to the 3'-end of the cloned DNA) and that having the sense strand to the target sequence by a second promoter (e.g., a promoter sequence linked to the 5'-end of the cloned DNA). The expressed sense and antisense strands hybridize to each other *in vivo* to generate a siRNA construct to silence a gene that comprises the target sequence.

15 Furthermore, two constructs (vectors) may be utilized to respectively produce the sense and anti-sense strands of a siRNA construct.

20 For introducing the vectors into a cell, transfection-enhancing agent can be used. FuGENE (Rochediagnostics), Lipofectamin 2000 (Invitrogen), Oligofectamin (Invitrogen), and Nucleofactor (Wako pure Chemical) are useful as the transfection-enhancing agent.

Pharmaceutical compositions for treating or preventing NSCLC

25 The present invention provides compositions for treating or preventing NSCLC comprising a compound selected by the present method of screening for a compound that alters the expression or activity of an NSCLC-associated gene.

30 When administering a compound isolated by the screening method of the present invention as a pharmaceutical for humans and other mammals, such as mice, rats, guinea-pig, rabbits, cats, dogs, sheep, pigs, cattle, monkeys, baboons or chimpanzees for treating a cell proliferative

disease (e.g., non-small cell lung cancer), the isolated compound can be directly administered or can be formulated into a dosage form using conventional pharmaceutical preparation methods. Such pharmaceutical formulations of the present compositions include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral, 5 (including intramuscular, sub-cutaneous and intravenous) administration, or for administration by inhalation or insufflation. The formulations are optionally packaged in discrete dosage units.

Pharmaceutical formulations suitable for oral administration include capsules, cachets or tablets, each containing a predetermined amount of the active ingredient. Formulations also 10 include powders, granules, solutions, suspensions or emulsions. The active ingredient is optionally administered as a bolus electuary or paste. Tablets and capsules for oral administration may contain conventional-excipients such as binding agents, fillers, lubricants, disintegrant or wetting agents. A tablet may be made by compression or molding, optionally with one or more formulational ingredients. Compressed tablets may be prepared by 15 compressing in a suitable machine the active ingredients in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made via molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be coated according to methods well known in the art. Oral fluid preparations may be in the form of, for 20 example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle prior to use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils) or preservatives. The tablets may optionally be formulated so as to provide slow or controlled release of the 25 active ingredient *in vivo*. A package of tablets may contain one tablet to be taken on each of the month. The formulation or dose of medicament in these preparations makes a suitable dosage within the indicated range acquirable.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the 30 formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately 35 prior to use. Alternatively, the formulations may be presented for continuous infusion.

Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration include suppositories with standard carriers such as cocoa butter or polyethylene glycol. Formulations for topical administration in the mouth, for example, buccally or sublingually, include lozenges, which contain the active ingredient in a flavored base such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a base such as gelatin, glycerin, sucrose or acacia. For intra-nasal administration of an active ingredient, a liquid spray or dispersible powder or in the form of drops may be used. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents.

For administration by inhalation the compositions are conveniently delivered from an insufflator, nebulizer, pressurized packs or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compositions may take the form of a dry powder composition, for example, a powder mix of an active ingredient and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules, cartridges, gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflators.

Other formulations include implantable devices and adhesive patches; which release a therapeutic agent.

When desired, the above-described formulations, adapted to give sustained release of the active ingredient, may be employed. The pharmaceutical compositions may also contain other active ingredients such as antimicrobial agents, immunosuppressants or preservatives.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

Preferred unit dosage formulations are those containing an effective dose, as recited below, of the active ingredient or an appropriate fraction thereof.

For each of the aforementioned conditions, the compositions, e.g., polypeptides and organic compounds are administered orally or via injection at a dose of from about 0.1 to about 250 mg/kg per day. The dose range for adult humans is generally from about 5 mg to about 17.5 g/day, preferably about 5 mg to about 10 g/day, and most preferably about 100 mg to about 3 g/day. Tablets or other unit dosage forms of presentation provided in discrete units may conveniently contain an amount which is effective at such dosage or as a multiple of the same, for instance, units containing about 5 mg to about 500 mg, usually from about 100 mg to about 500 mg.

The dose employed will depend upon a number of factors, including the age and sex of the subject, the precise disorder being treated, and its severity. Also the route of administration may vary depending upon the condition and its severity.

The present invention further provides a composition for treating or preventing NSCLC comprising active ingredient that inhibits the expression of any one of the gene selected from the group of KIF11, GHSR1b and NTSR1 genes. Such active ingredient can be an antisense-oligonucleotide, siRNA or ribozyme against the gene, or derivatives, such as expression vector, of the antisense-oligonucleotide, siRNA or ribozyme. The active ingredient may be made into an external preparation, such as liniment or a poultice, by mixing with a suitable base material which is inactive against the derivatives.

Also, as needed, the active ingredient can be formulated into tablets, powders, granules, capsules, liposome capsules, injections, solutions, nose-drops and freeze-drying agents by adding excipients, isotonic agents, solubilizers, preservatives, pain-killers and such. These can be prepared according to conventional methods for preparing nucleic acid containing pharmaceuticals.

Preferably, the antisense-oligonucleotide derivative, siRNA derivative or ribozyme derivative is given to the patient by direct application to the ailing site or by injection into a blood vessel so that it will reach the site of ailment. A mounting medium can also be used in the composition to increase durability and membrane-permeability. Examples of mounting mediums include liposome, poly-L-lysine, lipid, cholesterol, lipofectin and derivatives thereof.

The dosage of such compositions can be adjusted suitably according to the patient's condition and used in desired amounts. For example, a dose range of 0.1 to 100 mg/kg, preferably 0.1 to 50 mg/kg can be administered.

Another embodiment of the present invention is a composition for treating or preventing NSCLC comprising an antibody against a polypeptide encoded by any one of the genes selected

from the group of KIF11, GHSR1b and NTSR1 genes or fragments of the antibody that bind to the polypeptide.

Although there are some differences according to the symptoms, the dose of an antibody or fragments thereof for treating or preventing NSCLC is about 0.1 mg to about 100 mg per day, 5 preferably about 1.0 mg to about 50 mg per day and more preferably about 1.0 mg to about 20 mg per day, when administered orally to a normal adult (weight 60 kg).

When administering parenterally, in the form of an injection to a normal adult (weight 60 kg), although there are some differences according to the condition of the patient, symptoms of the disease and method of administration, it is convenient to intravenously inject a dose of about 10 0.01 mg to about 30 mg per day, preferably about 0.1 to about 20 mg per day and more preferably about 0.1 to about 10 mg per day. Also, in the case of other animals too, it is possible to administer an amount converted to 60 kg of body-weight.

15 The following examples are presented to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention.

20 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. Any patents, patent applications and publications cited herein are incorporated by reference.

BEST MODE FOR CARRYING OUT THE INVENTION

25 Materials and methods

(1) Patients and tissue samples

Primary NSCLC samples, of which 22 were classified as adenocarcinomas (ADCs), 14 as squamous-cell carcinomas (SCCs), and one as adenosquamous carcinoma, had been obtained earlier with informed consent from 37 patients (Kikuchi, T., Daigo, Y., Katagiri, T., Tsunoda, T., 30 Okada, K., Kakiuchi, S., Zembutsu, H., Furukawa, Y., Kawamura, M., Kobayashi, K., Imai, K., Nakamura, Y. "Expression profiles of non-small cell lung cancers on cDNA microarrays: identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs", Oncogene 22, 2192-2205 (2003)). Fifteen additional primary NSCLCs, including

seven ADCs and eight SCCs, were obtained along with adjacent normal lung tissue samples from patients undergoing surgery at our institutes.

(2) Cell lines

Twenty-one human NSCLC cancer cell lines were grown in monolayers in appropriate

5 medium supplemented with 5 or 10% fetal bovine serum (see Table 1).

Table 1

Cell line name	Medium
adenocarcinoma (ADC)	
A549	RPMI-1640 (10% FBS)
NCI-H23	RPMI-1640 (10% FBS)
NCI-H522	RPMI-1640 (10% FBS)
LC174	RPMI-1640 (10% FBS)
LC176	RPMI-1640 (10% FBS)
LC319	RPMI-1640 (10% FBS)
PC-3	DMEM (10% FBS)
PC-9	DMEM (10% FBS)
PC14	RPMI-1640 (10% FBS)
PC14-PE6	RPMI-1640 (10% FBS)
NCI-H1373	RPMI-1640 (10% FBS)
NCI-H1435	F12+DMEM (5%FBS)+EGF(+)
NCI-H1793	F12+DMEM (5%FBS)+Glu
SK-LU-1	DMEM (10% FBS)
BAC	NCI-H358
BAC	NCI-H1650
BAC	SW1573
squamous cell carcinoma (SCC)	
RERF-LC-A1	DMEM (10% FBS)
SW-900	Leibovitz's L-15 (10% FBS)
SK-MES-1	DMEM (10% FBS)
adenosquamous	NCI-H596
	RPMI-1640 (10% FBS)

(3) Semiquantitative RT-PCR analysis

Total RNA was extracted from cultured cells and clinical tissues using Trizol reagent (Life

10 Technologies, Inc.) according to the manufacturer's protocol. Extracted RNAs and normal human tissue poly(A) RNAs were treated with DNase I (Nippon Gene) and reverse-transcribed

using oligo(dT) primer and SuperScript II reverse transcriptase (Invitrogen). Semiquantitative RT-PCR experiments were carried out with the following synthesized gene-specific primers or with beta-actin (ACTB)-specific primers as an internal control:

KOC1, 5'-TAAATGGCTTCAGGAGACTTCAG-3' (SEQ.ID.NO.7) and
 5 5'-GGTTTAAATGCAGCTCCTATGTG-3' (SEQ.ID.NO.8);
 KIF11, 5'-CTGAACAGTGGGTATCTCCTTA-3' (SEQ.ID.NO.9) and
 5'-GATGGCTCTGACTTAGAGGTC-3' (SEQ.ID.NO.10);
 NMU, 5'-TGAAGAGATTCAAGAGTGGACGA-3' (SEQ.ID.NO.11) and
 5'-ACTGAGAACATTGACAACACAGG-3' (SEQ.ID.NO.12);
 10 NMU1R, 5'-AAGAGGGACAGGGACAAGTAGT-3' (SEQ.ID.NO.13) and
 5'-ATGCCACTGTTACTGCTTCAG-3' (SEQ.ID.NO.14);
 NMU2R, 5'-GGCTCTTACAACACTCATGTACCCA-3' (SEQ.ID.NO.15) and
 5'-TGATACAGAGACATGAAGTGAGCA-3' (SEQ.ID.NO.16);
 GHSR1a, 5'-TGGTGTTCGCCTTCATCCT-3' (SEQ.ID.NO.17) and
 15 5'-GAATCCCAGAACAGTCTGAACA-3' (SEQ.ID.NO.18);
 GHSR1b, 5'-ACGGTCCTCTACAGTCTCA-3' (SEQ.ID.NO.19) and
 5'-CACAGGGAGAGGATAGGA-3' (SEQ.ID.NO.20);
 NTSR1, 5'-AGTGGGCTCAGAGTCTAGCAAAT-3' (SEQ.ID.NO.21) and
 5'-TATTGAGAGATACACGGGGTTG-3' (SEQ.ID.NO.22);
 20 GHRL, 5'-TGAGCCCTGAACACCAGAGAG-3' (SEQ.ID.NO.23) and
 5'-AAAGCCAGATGAGCGCTTCTA-3' (SEQ.ID.NO.24);
 NTS, 5'-TCTTCAGCATGATGTGTTGTG-3' (SEQ.ID.NO.25) and
 5'-TGAGAGATTCATGAGGAAGTCTTG-3' (SEQ.ID.NO.26);
 ACTB, 5'-GAGGTGATAGCATTGCTTCG-3' (SEQ.ID.NO.27) and
 25 5'-CAAGTCAGTGTACAGGTAAGC-3' (SEQ.ID.NO.28).

PCR reactions were optimized for the number of cycles to ensure product intensity within the logarithmic phase of amplification.

(4) Northern-blot analysis

Human multiple-tissue blots (BD Biosciences Clontech) were hybridized with ³²P-labeled
 30 PCR products of KOC1, KIF11 and GHSR1. cDNA probes of KOC1, KIF11 and NMU were prepared by RT-PCR using primers similarly as above. Prehybridization, hybridization, and washing were performed according to the supplier's recommendations. The blots were autoradiographed with intensifying BAS screens (BIO-RAD) at room temperature (RT) for 30 to 168 hours.

35 **(5) RNA interference assay**

To prepare plasmid vector expressing short interfering RNA (siRNA), we amplified the genomic fragment of H1RNA gene containing its promoter region by PCR using a set of primers, 5'-TGGTAGCCAAGTGCAGGTTATA-3' (SEQ ID No: 47), and

5'- CCAAAGGGTTCTGCAGTTCA-3' (SEQ ID No: 48) and human placental DNA as a

5 template. The product was purified and cloned into pCR2.0 plasmid vector using a TA cloning kit according to the supplier's protocol (Invitrogen). The *Bam*HI and *Xba*I fragment containing *H1RNA* was into pcDNA3.1(+) between nucleotides 56 and 1257, and the fragment was amplified by PCR using

5'-TGCAGATCCAGAGCAGATTGACTGAGAGT-3' (SEQ ID No: 49) and

10 5'- CTCTATCTCGAGTGAGGCGGAAAGAACCA-3' (SEQ ID No: 50),

The ligated DNA became the template for PCR amplification with primers,

5'- TTTAAGCTTGAAGACCATTTTGAAAAAAC-3' (SEQ ID No: 51) and

5'-TTAAGCTTGAAGACATGGGAAAGAGTGGTCTCA-3' (SEQ ID No: 52).

15 The product was digested with *Hind*III, and subsequently self-ligated to produce psiH 1BX3.0 vector plasmid having a nucleotide sequence shown in SEQ ID NO: 53.

The DNA fragment encoding siRNA was inserted into the GAP at nucleotide 489-492 as indicated (-) in the following plasmid sequence (SEQ ID NO: 53).

20 GACGGATCGGGAGATCTCCCGATCCCCATGGTGCACCTCAGTACAATCTGCTCTGGATCCA
CTAGTAACGGCCGCCAGTGTGCTGAAATCGGCTTGGTAGCCAAGTGCAGGTTATAGGGAGC
TGAAGGGAAAGGGGGTCACAGTAGGTGGCATCGTCTCTGACTGCCGCCGGCGATGC
CGTCCCGCGATATTGAGCTCGAACCTCTGCCCTGCCGCCGGTGTCCGTCGCCGCC
CGCCGCCATGGAATTGAAACCGCTGACGTATCAACCGCTCCAAGGAATCGCAGGAGCAGT
GTCACTAGGCAGGAAACACCCAGCGCGTGCAGCCCTGGCAGGAAGATGGCTGTGAGGGAC
AGGGGAGTGGCGCCCTGCAATATTGATGTCGATGTGTTCTGGAAATCACCATAACG
15 GAAATGTCTTGGATTGGGAATCTTATAAGTTCTGTATGAGACCCTTCCC—TTTTGG
GAAAAA
25 GAAAAA
CCCCAGCACTTGGGAGGCCAGGCCGGATCACAAGGTCAAGGAGTCAGGAGGTGAGGACCATCCA
GGCTAACACGGTGAACACCCCCCCCACCTCTACTA
30 AAAAAAAATACAAAAAATTAGCCA
TTAGCCGGCGTGGTGGCGGCCATAATCCAGCTACTTGGGAGGCTGAAGCAGAATG
GCGTGAACCCGGAGGCCAGTGCAGTGCAGTGCAGGAGATCGCCGACTGCAITCCAGCCT
GGCGACAGAGCGAGTCTCA
35 AAAAAAAACCGAGTGGAAATGTGAAAAGCTCCGTGAAACT
GCAGAAACCCAAGCCGAATCTGAGATATCCATCACACTGGCGCCGCTGAGTGAGGCG
GAAAGAACCAAGCTGGGCTCTAGGGGTATCCCCACGCCCTGTAGCGGCCGATTAAGCG
CGGCCGGTGTGGTTACCGCAGCGTACCGCTACACTTGCACGCCCTAGCGGCCGC
40 TCCCTTCGCTTCTCCCTTCTGCCACGTTGCGCCGGCTTCCCGTCAAGCTCTAAAT
CGGGGGCTCCCTTIAGGGTCCGATTAGTGTCTTACGGCACCTCGACCCCAAAAAACTG
TTAGGGTGATGGTTCACGTAGTGGGCCATGCCCTGATAGACGGTTTTCGCCCTTGACGTT
GGAGTCCACGTTCTTAATAGTGGACTCTGTTCAAAACTGGAACAAACACTCAACCCCTATCT
CGGTCTATTCTTGTATTATAAGGGATTGCGCATTGCCCTATTGGTTAAAAAATGAGCT
GATTAAACAAAATTAAACGCGAATTATTCTGTGGAATGTGTCAGTTAGGGTGTGGAAA

5 GTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCA
 GGTGTGAAAGTCCCCAGGCTCCCCAGGCAGAGAAGTATGCAAAGCATGCATCTCAAITA
 10 GTCAGCAACCATACTCCGCCCTAACTCCGCCATCCGCCCTAACTCCGCCAGTCCG
 CCCATTCTCCGCCCTAGGCTGACTAATTATTTATTCAGAGGGCCAGGCCGCTCTG
 CCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCCTTTGGAGGCCCTAGGCCTTGGAAAAAG
 CTCCCGGAGCTTGTATATCCATTTCGGATCTGATCAAGAGACAGGATGAGGATGTTTCGC
 ATGATTGAACAAGATGGATTCGACGCCAGGTTCTCCGCCGCTGGGTGGAGAGGGCTATTCCG
 CTATGACTGGGACAACAGACAATCCGCTGCTGATGCCGCCGTTCCGGCTGTCAGCGC
 AGGGCGCCCGTCTTTGTCAAGACCGACCTGTCGGTGCCTGAATGAACGTGAGGA
 15 CGAGGCAGCGCGCTATCGTGGCTGGCACGACGGCGTCCCTGGCAGCTGTGCTCGAC
 GTTGTCACTGAAGCGGGAGGGACTGGCTGCTATTGGCGAAGTGGCCGGGAGGATCTCC
 TGTCATCTCACCTTGCTCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCCGCGCTGC
 ATACCGCTTGATCCGGTACCTGCCATTGACCAAGCGAAACATCGCATCGAGCGAGCA
 CGTACTCGGATGGAAGCCGCTTGTGATCAGGATGATCTGGACGAAGAGCATAGGGCT
 20 CGCGCCAGCCGAACTGTCGCCAGGCTCAAGGCCGATGCCGACGGCGAGGATCTCGC
 GTGACCCATGGCGATGCCGCTTGCGAATATCATGGTGGAAAATGCCGCTTCTGGATTC
 ATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCCTGGTACCCGTGA
 TATTGCTGAAGAGCTGGCGCGAATGGCTGACCGCTTCTCGTCTTACGGTATCGCCG
 CTCCCGAATCGCAGCGCATGCCCTTATGCCCTTGTACGAGTTCTGAGCGGGACTCT
 25 GGGGTCGAAATGACCGACCAAGCGACGCCAACCTGCATCACGAGATTGATTCACC
 GCCGCCCTATGAAAGGTTGGCTCGGAATCGTCTTCCGGGACGCCGGCTGGATGATCCT
 CCAGCGGGGATCTCATGCTGGAGCTTCCGCCACCCAACTTGTATTGAGCTTATAA
 TGGTACAATAAAAGCAATAGCATCACAATTACAAATAAAGCATTTTCACTGCAITC
 TAGTGTGGTTGTCCAACATCATCAATGTTATCATGCTGTATACCGTCGACCTCTAGC
 30 TAGAGCTGGCGTAATCATGGTCATAGCTGTTCTGTGAAATTGTTATCCGCTACAATTC
 CACACAACATACGAGCGGAAGCATAAAAGTGTAAAGCTGGGTGCTTAATGAGTGAGCTA
 ACTCACATTAATGCGTTGCGCTACTGCCGCTTCCAGCTGGGAAACCTGTCGTGCCAGC
 TGCAATTGAATCGGCCAACCGCGGGAGAGGCGGTTGCGTATTGGCGCTTCCGCT
 TCCCTGCTCACTGACTCGCTCGCTCGTCTGGCTGCGGAGCGGTATCAGCTCACTC
 35 AAAGGCGGTAATACGGTTATCCACAGAACAGGGATAACCGCAGGAAAGAACATGTGAGCA
 AAAGGCCAGCAAAAGGCCAGGAACCGTAAAAGGCCGCTGCTGGCTTTCCATAGGC
 TCCGCCCTGACGAGCATCACAAAATGACGCTCAAGTCAGAGGTGGCGAAACCCGAC
 AGGACTATAAAGATAACCGAGCGTTCCCTGGAAGCTCCCTGCGCTCTCTGGTCCGA
 CCCTGCCCTACCGGATACTGTCCGCCCTTCTCCCTGGAAAGCGTGGCGCTTCTCAT
 40 GCTCACGCTGTAGGTATCTAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGCTGTGCA
 GAACCCCCCGTTCAAGCCCACCGCTGGTAGCGGTTTTGTTGCAAGCAGCAGATTACGCGC
 GGTAAGACACGACTATGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGG
 TATGTAAGCGGTGCTACAGAGTTCTGAAGTGGTGGCTAACTACGGCTACACTAGAAC
 AGTATTGTTATCGCCTGCTGAAGCCAGTTACCTTCCGGAAAAAGAGTTGAGCTT
 45 GATCCGGCAAACAAACCACCGCTGGTAGCGGTTTTGTTGCAAGCAGCAGATTACGCGC
 AGAAAAAAAGGATCTCAAGAAGATCTTGTATCTTCTACGGGCTGACGCTCAGTGGAA
 CGAAAACCTACGTTAAGGATTTGGTCACTGAGATTATCAAAAGGATCTCACCTAGATCT
 TTTAAATTAAAGTAAAGTTAAATCAATCTAAAGTATATGAGTAAACTGGTCTGACAGT
 TACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGCTATTTCGTTCATCC
 50 CCTGACTCCCCGCTGTTAGATAACTACGATAACGGGAGGGCTTACCATCTGGCCCCAGTGC
 GCAATGATACCGCGAGACCCACGCTACCGGCTCCAGATTATCAGCAATAAACCGCCAGC
 CGGAAGGGCCGAGCGCAGAAGTGGCTGCAACTTATCCGCTCCATCCAGTCTATTAA
 GTTGCCGGAAAGCTAGAGTAAGTAGTTGCGCTTAATAGTTGCGCAACGTTGTC
 GCTACAGGCATCGTGGTGTACGCTCGTCTGGTATGGCTTCACTCAGCTCCGGTCC
 CGATCAAGGCAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCC
 TCCGAATCGTTGTCAGAAGTAAGTTGGCGCAGTGTATCCTACATGGTTATGGCAGCA
 TAATTCTTACTGTCACTGCCATCCGTAAGATGCTTTCTGTGACTGGTAGTACTCAACCAA

5 GTCATTCTGAGAATAGTGTATGCGGGGACCGAGTTGCTCTGCCCGCGTCAATACGGGATAA
 TACCGCGCCACATAGCAGAACITAAAGTGTCTCATTTGGAAAACGTTCTCGGGGCGAA
 AACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAAAC
 TGATCTTCAGCATTTCACCTTCACAGCGTTCTGGGTGAGCAAAAACAGGAAGGCAAAA
 TGCGCAAAAAGGGAAATAAGGGGACACCGAAATGTTGAATACTCATACTCTCCCTTTTC
 AATATTAATGAAAGCATTATCAGGGTATTGTCTCATGAGCGGATACATAATTGAATGTATTAG
 AAAAATAACAAATAGGGGTCGGCGCACATTCCCCGAAAAGTGCCACCTGACGTC

10 Using 30 μ l of Lipofectamine 2000 (Invitrogen), 10 μ g of siRNA-expression vector were
 transfected into NSCLC cell lines, A549 and LC319, both endogenously over-expressing KOC1,
 KIF11, NMU, GHSR1b, and NTSR1. More than 90% of the transfected cells expressed the
 synthetic siRNAs, and endogenous expression of target genes (KIF11, GHSR, or NTSR1) in
 these cells was effectively suppressed. The transfected cells were cultured for five days in the
 presence of appropriate concentrations of geneticin (G418), and then, cell numbers and viability
 15 were measured by Giemsa staining and triplicate MTT assays. The target sequences of the
 synthetic oligonucleotides for RNAi were as follows: control 1 (EGFP: enhanced green
 fluorescent protein (GFP) gene, a mutant of *Aequorea victoria* GFP),
 20 5'-GAAGCAGCACGACTTCTTC-3' (SEQ.ID.NO.29); control 2 (Luciferase: *Photinus pyralis*
 luciferase gene), 5'-CGTACCGGAATACTTCGA-3' (SEQ.ID.NO.30); control 3 (Scramble:
 chloroplast *Euglena gracilis* gene coding for 5S and 16S rRNAs),
 25 5'-GCGCGCTTGTAGGATTG-3' (SEQ.ID.NO.31);
 siRNA-KIF11-1 (#1), 5'-GTTAGTGTACGAACGGAG-3' (SEQ.ID.NO.32);
 siRNA-KIF11-2 (#2), 5'-GTGTCTCTGTTGGAGATCT-3' (SEQ.ID.NO.33);
 siRNA-KIF11-3 (#3), 5'-GAAGGCAGTTGACCAACAC-3' (SEQ.ID.NO.34);
 30 siRNA-KIF11-4 (#4), 5'-GAGACTGAACAGAGATGTG-3' (SEQ.ID.NO.35);
 siRNA-KIF11-5 (#5), 5'-GCCAATGTTGTGAGGCTTC-3' (SEQ.ID.NO.36);
 siRNA-GHSR-1 (si-GHSR-1), 5'-CCTCTACCTGTCCAGCATG-3' (SEQ.ID.NO.37);
 siRNA-GHSR-2 (si-GHSR-2), 5'-GCTGGTCATCTCGTCATC-3' (SEQ.ID.NO.38);
 si-RNA-NTSR1-1 (si-NTSR1-1), 5'-GTTCATCAGCGCCATCTGG-3' (SEQ.ID.NO.39);
 35 si-RNA-NTSR1-2 (si-NTSR1-2), 5'-GGTCGTACAGGTCAAC-3' (SEQ.ID.NO.40).

The oligonucleotides used for these siRNAs are shown below. Each constructs were prepared
 by cloning the following double-stranded oligonucleotide into the BbsI site in the psiH1BX3.0
 vector. The corresponding nucleotide position relative to the KIF11, GHSR1b, and NTSR1
 nucleic acid sequence of SEQ ID NOs:1, 3 and 5 are listed for each oligonucleotide sequence.
 35 Each oligonucleotide is a combination of a sense nucleotide sequence and an antisense
 nucleotide sequence of the target sequence of KIF11, GHSR1b, and NTSR1. The nucleotide
 sequences of the hairpin loop structure of each siRNAs are also shown below. (endonuclease

recognition sites are eliminated from each hairpin loop structure sequence).

KIF11 si1 288-306 (for the target sequence of gttagtgtac gaactggag/ SEQ ID NO:32)

(insert F) Tccc gttagtgtacgaactggag ttcaagaga ctccagttcgtaactaac/SEQ ID NO:54

(insert R) Aaaa gttagtgtacgaactggag tctcttcaa ctccagttcgtaactaac/SEQ ID NO:55

5 (hairpin) gttagtgtacgaactggag ttcaagaga ctccagttcgtaactaac/SEQ ID NO:56

KIF11 si2 612-630 (for the target sequence of gtgtctctgt tggagatct/ SEQ ID NO:33)

(insert F) Tccc gtgtctctgt tggagatct ttcaagaga agatctccaacagagacac/SEQ ID NO:57

(insert R) Aaaa gtgtctctgt tggagatct tctcttcaa agatctccaacagagacac/SEQ ID NO:58

10 (hairpin) gtgtctctgt tggagatct ttcaagaga agatctccaacagagacac/SEQ ID NO:59

KIF11 si3 1700-1718 (for the target sequence of gaaggcagtt gaccaacac/ SEQ ID NO:34)

(insert F) Tccc gaaggcagtt gaccaacac ttcaagaga gtgtggtaactgccttc/SEQ ID NO:60

(insert R) Aaaa gaaggcagtt gaccaacac tctcttcaa gtgtggtaactgccttc/SEQ ID NO:61

15 (hairpin) gaaggcagtt gaccaacac ttcaagaga gtgtggtaactgccttc/SEQ ID NO:62

KIF11 si4 2580-2598 (for the target sequence of gagactgaac agagatgt/ SEQ ID NO:35)

(insert F) Tccc gagactgaac agagatgt ttcaagaga cacatctctgtcagtc/SEQ ID NO:63

(insert R) Aaaa gagactgaac agagatgt tctcttcaa cacatctctgtcagtc/SEQ ID NO:64

20 (hairpin) gagactgaac agagatgt ttcaagaga cacatctctgtcagtc/SEQ ID NO:65

KIF11 si5 2695-2713 (for the target sequence of gccaatgtg tgaggcttc/ SEQ ID NO:36)

(insert F) Tccc gccaatgtg tgaggcttc ttcaagaga gaagcctcacaacattggc/SEQ ID NO:66

(insert R) Aaaa gccaatgtg tgaggcttc tctcttcaa gaagcctcacaacattggc/SEQ ID NO:67

25 (hairpin) gccaatgtg tgaggcttc ttcaagaga gaagcctcacaacattggc/SEQ ID NO:68

GHSR1b si1 237-255 (for the target sequence of cctctacactg tccagcatg/ SEQ ID NO:37)

(insert F) Tccc cctctacactg tccagcatg ttcaagaga catgtggacaggttagagg/SEQ ID NO:69

(insert R) Aaaa cctctacactg tccagcatg tctcttcaa catgtggacaggttagagg/SEQ ID NO:70

30 (hairpin) cctctacactg tccagcatg ttcaagaga catgtggacaggttagagg/SEQ ID NO:71

GHSR1b si2 483-501 (for the target sequence of gctggtcatc ttctgtcatc/ SEQ ID NO:38)

(insert F) Tccc gctggtcatc ttctgtcatc ttcaagaga gatgacgaagatgaccagc/SEQ ID NO:72

(insert R) Aaaa gctggtcatc ttctgtcatc tctcttcaa gatgacgaagatgaccagc/SEQ ID NO:73

35 (hairpin) gctggtcatc ttctgtcatc ttcaagaga gatgacgaagatgaccagc/SEQ ID NO:74

NTSR1 si1 933-951 (for the target sequence of gttcatcgc gccatctgg/ SEQ ID NO:39)

(insert F) Tccc gttcatcgc gccatctgg ttcaagaga ccagatggcgctatgaac/SEQ ID NO:75

(insert R) Aaaa gttcatcgc gccatctgg tctcttgg ccagatggcgctatgaac/SEQ ID NO:76

5 (hairpin) gttcatcgc gccatctgg ttcaagaga ccagatggcgctatgaac/SEQ ID NO:77

NTSR1 si2 1074-1092 (for the target sequence of ggtcgatata caggtaaac/ SEQ ID NO:40)

(insert F) Tccc ggtcgatata caggtaaac ttcaagaga gttgacctgtatgacgacc/SEQ ID NO:78

(insert R) Aaaa ggtcgatata caggtaaac tctcttgg gttgacctgtatgacgacc/SEQ ID NO:79

10 (hairpin) ggtcgatata caggtaaac ttcaagaga gttgacctgtatgacgacc/SEQ ID NO:80

To validate RNAi system of the present invention, individual control siRNAs (EGFP, Luciferase, and Scramble) were initially confirmed using semiquantitative RT-PCR to decrease the expression of the corresponding target genes that had been transiently transfected into

15 COS-7 cells. Down-regulation of KIF11, GHSR1b, and NTSR1 expression by their respective siRNAs (si-KIF11-1, si-KIF11-2, si-KIF11-3, si-KIF11-4, si-KIF11-5, si-GHSR-1, si-NTSR1-1, and si-NTSR1-2), but not by controls, was confirmed with semiquantitative RT-PCR in the cell lines used for this assay.

(6) Co-immunoprecipitation and MALDI-TOF mass spectrometry

20 Human lung cancer cell line LC319 cells were transfected with bilateral-tagged pCAGGS-n3FH (NH2-terminal FLAG, COOH-terminal HA)-KOC1 expression vector or empty vector (mock transfection). Cells were extracted in IP-buffer (0.5% NP-40, 50 mM Tris-HCl, 150 mM NaCl, and protease inhibitor) for 30 min on ice. Extracts were centrifuged at 14,000 rpm for 15 min, and supernatants were subjected to immunoprecipitation using

25 anti-Flag M2 agarose and anti-HA beads (Sigma-Aldrich) for 1-2 hours. The beads were washed six times with IP-buffer, and protein was eluted by boiling the beads in Laemmli sample buffer after removing the final wash fraction. The eluted protein was resolved by SDS-PAGE and stained with silver staining. A 120 kDa-band was extracted by gel extraction, and used for mass spectrometric sequencing using MALDI-TOF mass spectrometry. This

30 analysis identified KIF11 as the 120 kDa product.

To confirm the interaction between KOC1 and KIF11, A549 cells were transiently co-transfected with Flag-tagged KIF11 and myc-tagged KOC1 and the cells were immunoprecipitated with anti-Flag M2 agarose. Subsequently, the cells were immunoblotted with anti-myc antibody (9E10; Santa Cruz). Next, using the same combination of vectors and 35 cells, the cells were immunoprecipitated with anti-myc agarose (SIGMA) and immunoblotted

with anti-Flag M2 antibody.

To further confirm this interaction, A549 cells were transiently co-transfected with Flag-tagged KIF11 and myc-tagged KOC1, and co-localization of FITC-labeled KIF11 and rhodamine-labeled KOC1 mainly in the cytoplasm was detected by immunocytochemical 5 staining using FITC-labeled anti-FLAG antibody and rhodamine-labeled anti-myc antibody, as described below.

(7) Immunocytochemistry

A549 cells grown on coverslips were cultured for 24 hours after passage, and were co-transfected with Flag-tagged KIF11 and myc-tagged KOC1. After 24-hours incubation, the 10 cells were fixed with acetone/methanol (1:1) for 5 min on ice, blocked in CAS BLOCK (ZYMED) for 7 min at RT, and then incubated with rabbit anti-Flag polyclonal antibody (SIGMA) for 1 hour at RT. The fixed cells were washed 3 times with PBS, reacted with anti-rabbit IgG-FITC for 1 hour at RT. Then the cells were blocked again, and incubated with anti-myc antibody (9E10; Santa Cruz) for 1 hour at RT. Finally anti-mouse IgG-rhodamin was 15 applied to the cells for 1 hour at RT. The cells were viewed on a Leica TCS SP2-AOBS confocal microscope.

(8) Ligand-receptor binding assay

To identify direct binding of NMU-25 to its candidate receptors, GHSR1a, GHSR1b and NTSR1, the following experiments were performed. The entire coding region of each receptor 20 gene was amplified by RT-PCR using primers GHSR1a (5'-GGAAATTCCATGTGGAACCGCGACGCCAGCGAA-3' (SEQ.ID.NO.41) and 5'-CGCGGATCCCGGTATTAAATACTAGATTCTGTCCAGGCC-3' (SEQ.ID.NO.42)), GHSR1b (5'-GGAAATTCCATGTGGAACCGCGACGCCAGCGAA-3' (SEQ.ID.NO.43) and 5'-CGCGGATCCCGGGAGAGAAGGGAGAAGGCACAGGGGA-3' (SEQ.ID.NO.44)), and 25 NTSR1 (5'-GGAATTCCATGCGCCTAACAGCTCCGCGCCGGAA-3' (SEQ.ID.NO.45) and 5'-CGCGGATCCCGGGTACAGCGTCTCGCGGGTGGCATTGCT-3' (SEQ.ID.NO.46)). The products were digested with *Eco*R1 and *Bam*H1 and cloned into appropriate sites of p3XFLAG-CMV10 vector (Sigma-Aldrich Co.). COS-7 cells were transfected with GHSR1b or NTSR1 expression plasmids using FuGENE6, as described above. Transfected COS-7 cells 30 were cultured with 0.5 μ M rhodamine-labeled NMU-25 peptide (NMU-25-rhodamine: Phoenix Pharmaceuticals, Inc.) for 12 hours, washed five times in PBS(-), and fixed in 4% paraformaldehyde solution for 60 min at room temperature. Then the cells were incubated with antibodies to FLAG-tagged GHSR1a, GHSR1b, or NTSR1 proteins (Sigma-Aldrich Co.), 35 stained with a goat anti-mouse secondary antibody conjugated to FITC (Cappel) and viewed under laser-confocal microscopy (TCS SP2 AOBS: Leica Microsystems). In addition, three

different negative controls were prepared for this assay: 1) non-transfected COS-7 cells without addition of NMU-25-rhodamine; 2) non-transfected COS-7 cells treated with NMU-25-rhodamine; and 3) COS-7 cells transfected with GHSR1a, GHSR1, or NTSR1 without NMU-25-rhodamine. COS-7 cells transfected with a known NMU receptor 5 (NMU1R) served as a positive control for the assay.

To confirm the binding of NMU-25 to the candidate receptors, flow-cytometric analysis was performed using the same series of COS-7 cells. Specifically, COS-7 cells were plated at a density of 1 X 10⁵ cells/100-mm dish and transfected with either GHSR1b, NTSR1, or NMU1R expression vectors; 24 hours after transfection, cells were incubated with 0.5 μ M 10 NMU-25-rhodamine for 12 hours, washed, trypsinized, collected in PBS, and washed once more in PBS. The population of cells binding to rhodamine-labeled NMU-25 was determined by flow cytometry.

Results

(1) Identification of KIF11 as a protein interacting with KOC1

LC319 cells transfected with n3FH-pCAGGS-KOC1 vector were extracted and immunoprecipitated with anti-Flag M2 monoclonal antibody, and subsequently immunoprecipitated with anti-HA monoclonal antibody. The protein complex including KOC1 was stained with silver staining on SDS-PAGE gel. A 120 kDa band that was absent in 20 mock transfection was extracted and determined to be KIF11 (NM_004523;SEQ.ID.NO.1) by Mass spectrometric sequencing. (Fig. 1).

(2) Confirmation of interaction between KOC1 and KIF11

The A549 cells co-transfected with Flag-tagged KIF11 and myc-tagged KOC1, the cells transfected with either KIF11 or KOC1, and the non-transfected cells were immunoprecipitated 25 with anti-Flag M2 agarose and subsequently immunoblotted with anti-myc antibody. In contrast, the same series of A549 cells were immunoprecipitated with anti-myc agarose and immunoblotted with anti-Flag M2 antibody. A single band was found only when both constructs were co-transfected (Fig. 2a). Immunocytochemistry showed that FLAG-tagged FITC-labeled KIF11 co-localized in cytoplasm of A549 with myc-tagged rhodamine-labeled 30 KOC1 (Fig. 2b).

(3) KIF11 expression in NSCLC

Validation of KIF11 expression was performed in primary NSCLCs (clinical samples) and lung cancer cell lines. Increased KIF11 expression was confirmed in 12 of 16 NSCLC cases (5 of 8 ADCs and in 7 of 8 SCCs (Fig. 3). In addition, up-regulation of KIF11 were observed 35 in 14 of the 15 NSCLC cell lines (Fig. 3). The expression pattern of KIF11 in NSCLCs was

significantly concordant with that of KOC1.

(4) KIF11 expression in normal human tissues

Northern blotting with KIF11 cDNA as a probe identified 4.5- and 5.5-kb transcripts as very weak bands, only seen in placenta, testis, and bone marrow, among the 23 normal human

5 tissues examined. The expression pattern of KIF11 in normal human tissues was significantly correlated with that of KOC1 (Fig. 4).

(5) Inhibition of growth of NSCLC cells by siRNA against KIF11

Transfection of either siRNA plasmids for KIF11 into A549 (Fig. 5a) or LC319 (data not shown) cells suppressed mRNA expression of the KIF11 in comparison to cells containing any 10 of the three control siRNAs and mock transfection. In accordance with the reduced mRNA expression, A549 and LC319 cells showed significant decreases in cell viability and colony numbers measured by MTT (Fig. 5b) and colony-formation assays (data not shown).

(6) Screening of candidate receptors for NMU in NSCLC

Two known NMU receptors, NMU1R (FM3/GPR66) and NMU2R (FM4) play important 15 roles in energy homeostasis(Fujii, R. et al. "Identification of neuromedin U as the cognate ligand of the orphan G protein-coupled receptor FM-3." *J. Biol. Chem.* 275: 21068-21074 (2000); Howard, A.D. et al. "Identification of receptors for neuromedin U and its role in feeding." *Nature* 406: 70-74 (2000); Funes, S. et al. "Cloning and characterization of murine neuromedin U receptors." *Peptides* 23: 1607-1615 (2002)). NMU1R is present in many 20 peripheral human tissues (Fujii, R. et al. "Identification of neuromedin U as the cognate ligand of the orphan G protein-coupled receptor FM-3." *J. Biol. Chem.* 275: 21068-21074 (2000); Howard, A.D. et al. "Identification of receptors for neuromedin U and its role in feeding." *Nature* 406: 70-74 (2000); Funes, S. et al. "Cloning and characterization of murine neuromedin U receptors." *Peptides* 23: 1607-1615 (2002)), but NMU2R is located only in brain. To 25 investigate whether NMU1R and NMU2R genes were expressed in NSCLCs, expression of these NMU receptors were analyzed in normal human brain and lung, in NSCLC cell lines, and in clinical tissues by semiquantitative RT-PCR experiments. Neither NMU1R nor NMU2R expression was detected in any of the cell lines or clinical samples examined, although NMU1R was expressed in lung and NMU2R in brain (data now shown), suggesting that NMU could be 30 mediating growth of lung-cancer cells through interaction with other receptor(s).

Since NMU2R and NMU1R were originally isolated as homologues of known neuropeptide GPCRs, unidentified NMU receptor(s) were speculated to be members of the same family that would show some degree of homology to NMU1R/NMU2R. Hence, candidate NMU receptors were searched using the BLAST program. The results and their high expression

levels in NSCLCs in the expression profile data of the present inventors indicated GHSR1b (NM_004122;SEQ ID NOs: 3 and 4) and NTSR1 (NM_002531;SEQ ID NOs: 5 and 6) to be good candidates. GHSR has two transcripts, types 1a and 1b. The full-length human type 1a cDNA encodes a predicted polypeptide of 366 amino acids with seven transmembrane domains, 5 a typical feature of G protein-coupled receptors. A single intron divides its open reading frame into two exons encoding transmembrane domains 1-5 and 6-7, thus placing the GHSR1a into the intron-containing class of GPCRs. Type 1b is a non-spliced mRNA variant transcribed from a single exon that encodes a polypeptide of 289 amino acids with five transmembrane domains. The semiquantitative RT-PCR analysis using specific primers for 10 each variant indicated that GHSR1a was not expressed in NSCLCs. On the other hand, GHSR1b and NTSR1 were expressed at a relatively high level in some NSCLC cell lines, but not at all in normal lung (Fig. 7). The GHSR1b product has 46% homology to NMU1R, and NTSR1 encodes 418 amino acids with 47% homology to NMU1R.

(7) Identification of candidate receptors for NMU in NSCLC

15 To confirm direct interaction between NMU and GHSR1b/NTSR1, COS-7 cells were transiently transfected with plasmids designed to express FLAG-tagged GHSR1b or NTSR1, and cultured in the presence of rhodamine-labeled NMU-25. Then the localization of FLAG-tagged GHSR1b/NTSR1 and NMU-25-rhodamine in the cells were examined using anti-FLAG antibodies conjugated to FITC, and found that NMU-25 and either of both receptors 20 were located together on the cell membrane (Fig. 8a). Co-localization of NMU-25 with these receptors was not observed in control assays involving either of the following ligand/cell combinations: 1) NMU-25-rhodamine incubated with COS-7 cells that were not transfected with either of the receptor plasmids; 2) non-transfected COS-7 cells incubated without NMU-25-rhodamine; and 3) COS-7 cells transfected with either of the receptor plasmids, but 25 incubated without NMU-25-rhodamine. The result was confirmed by flow cytometry, which revealed binding of rhodamine-labeled NMU-25 to the surface of COS-7 cells that expressed either of the two receptors (Fig. 8b).

(8) GHSR1b expression in normal human tissues

As the expression of GHSR1b in normal human tissues was not precisely reported at the time, 30 the distribution of GHSR1b was determined using human multiple tissue Northern-blot. Northern blotting with GHSR1b cDNA as a probe identified a 0.9-kb transcript as a very weak signal band in comparison with a 1.1-kb transcript GHSR1a, seen in the heart, liver, skeletal muscle, pancreas, and stomach, among the 23 normal human tissues examined (Fig. 9).

(9) Inhibition of growth of NSCLC cells by siRNA against GHSR/NTSR1

Furthermore, the biological significance of the NMU-receptor interaction in pulmonary carcinogenesis was examined using plasmids designed to express siRNA against GHSR or NTSR1 (si-GHSR-1, si-NTSR1-1, and si-NTSR1-2). Transfection of either of these plasmids into A549 or LC319 cells suppressed expression of the endogenous receptor in comparison to 5 cells containing any of the three control siRNAs (Fig. 10a). In accordance with the reduced expression of the receptors, A549 and LC319 cells showed significant decreases in cell viability (Fig. 10b) and numbers of colonies (data not shown). These results strongly supported the possibility that NMU, by interaction with GHSR1b and NTSR1, might play a very significant role in development/progression of NSCLC.

10

Industrial Applicability

The expression of human genes KIF11, GHSR1b and NTSR1 are markedly elevated in non-small cell lung cancer (NSCLC) as compared to normal lung tissues. Accordingly, these 15 genes may serve as diagnostic markers of NSCLC and the proteins encoded thereby may be used in diagnostic assays of NSCLC.

The present inventors have also shown that the expression of KIF11, GHSR1b or NTSR1 promotes cell growth whereas cell growth is suppressed by small interfering RNAs corresponding to KIF11, GHSR1b or NTSR1 gene. These findings suggest that each of KIF11, GHSR1b and NTSR1 proteins stimulate oncogenic activity. Thus, each of these oncoproteins 20 is a useful target for the development of anti-cancer pharmaceuticals. For example, agents that block the expression of KIF11, GHSR1b or NTSR1, or prevent its activity may find therapeutic utility as anti-cancer agents, particularly anti-cancer agents for the treatment of NSCLC. Examples of such agents include antisense oligonucleotides, small interfering RNAs, and 25 ribozymes against the KIF11, GHSR1b or NTSR1 gene, and antibodies that recognize KIF11, GHSR1b or NTSR1 polypeptide.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope of the invention.

What is claimed is:

1. A method of diagnosing non-small cell lung cancer (NSCLC) or a predisposition to developing non-small cell lung cancer in a subject, comprising determining the expression level of a non-small cell lung cancer-associated gene in a biological sample derived from the subject, wherein an increase of said expression level compared to a normal control level of said gene indicates that said subject suffers from or is at risk of developing NSCLC, wherein said NSCLC-associated gene is selected from the group consisting of KIF11, GHSR1b, and NTSR1.
2. The method of claim 1, wherein said increase is at least 10% greater than said normal control level.
3. The method of claim 1, wherein said method further comprises determining the expression level of a plurality of NSCLC-associated genes.
4. The method of claim 1, wherein said expression level is determined by any one method selected from the group consisting of:
 - (1) detecting mRNA of the NSCLC-associated gene;
 - (2) detecting protein encoded by the NSCLC-associated gene; and
 - (3) detecting the biological activity of protein encoded by the NSCLC-associated gene.
5. The method of claim 1, wherein said expression level is determined by detecting hybridization of an NSCLC-associated gene probe to a gene transcript of said patient-derived biological sample.
6. The method of claim 5, wherein said hybridization step is carried out on a DNA array.
7. The method of claim 1, wherein said biological sample comprises sputum or blood.
8. A NSCLC reference expression profile, comprising a gene expression pattern of two or more genes selected from the group consisting of KIF11, GHSR1b, and NTSR1.
9. A kit comprising two or more detection reagents which detect the expression of one or more genes selected from the group consisting of KIF11, GHSR1b, and NTSR1.
10. An array comprising two or more polynucleotides which bind to one or more genes selected from the group consisting of KIF11, GHSR1b, and NTSR1.
11. A method of identifying a compound that inhibits the expression level of an NSCLC-associated gene, comprising the steps of:
 - (1) contacting a test cell expressing said NSCLC-associated gene with a test compound;

(2) detecting the expression level of said NSCLC-associated gene; and
(3) determining the compound that suppresses said expression level compared to a normal control level of said gene as an inhibitor of said NSCLC-associated gene
wherein said NSCLC-associated gene is selected from the group consisting of KIF11,
5 GHSR1b, and NTSR1.

12. The method of claim 11, wherein said test cell is NSCLC cell.

13. A method of screening for a compound for treating or preventing NSCLC, said method comprising the steps of:
(1) contacting a test compound with a polypeptide selected from the group consisting of
10 KIF11, GHSR1b, and NTSR1;
(2) detecting the binding activity between the polypeptide and the test compound; and
(3) selecting a compound that binds to the polypeptide.

14. A method of screening for a compound for treating or preventing NSCLC, said method comprising the steps of:
15 (a) contacting a test compound with a polypeptide encoded by a polynucleotide selected from the group consisting of KIF11, GHSR1b, and NTSR1;
(b) detecting the biological activity of the polypeptide of step (a); and
(c) selecting a compound that suppresses the biological activity of the polypeptide selected
20 from the group consisting of KIF11, GHSR1b, and NTSR1 in comparison with the biological activity detected in the absence of the test compound.

15. The method of claim 14, wherein said biological activity is cell proliferative activity.

16. A method of screening for a compound for treating or preventing NSCLC, said method comprising the steps of:
(1) contacting a test compound with a cell expressing one or more marker genes, wherein
25 the one or more marker genes is selected from the group consisting of KIF11, GHSR1b, and NTSR1; and
(2) selecting a compound that reduces the expression level of one or more marker genes selected from the group consisting of KIF11, GHSR1b, and NTSR1.

17. The method of claim 16, wherein said cell is NSCLC cell.

30 18. A method of screening for compound for treating or preventing NSCLC, said method comprising the steps of:
(1) contacting a test compound with a cell into which a vector comprising the

transcriptional regulatory region of one or more marker genes and a reporter gene that is expressed under the control of the transcriptional regulatory region has been introduced, wherein the one or more marker genes are selected from the group consisting of KIF11, GHSR1b, and NTSR1;

5 (2) measuring the activity of said reporter gene; and
(3) selecting a compound that reduces the expression level of said reporter gene, as compared to a control.

19. A method of screening for a compound for treating or preventing NSCLC, said method comprising the steps of:

10 (1) contacting a KIF11 polypeptide or functional equivalent thereof with KOC1 in the presence of a test compound;
(2) detecting the binding between the polypeptide and KOC1; and
(3) selecting the test compound that inhibits the binding between the polypeptide and KOC1.

15 20. A method of screening for a compound for treating or preventing NSCLC, said method comprising the steps of:

(1) contacting a GHSR1b or NTSR1 polypeptide, or functional equivalent thereof with NMU in the existence of a test compound;
(2) detecting the binding between the polypeptide and NMU; and
(3) selecting the test compound that inhibits the binding between the polypeptide and NMU.

21. The method of claim 20, wherein the polypeptide is expressed on the surface of a living cell.

25 22. The method of claim 21, wherein the binding between the polypeptide and NMU is detected by any one method select from the group consisting of:

(1) detecting the concentration of calcium or cAMP in the cell;
(2) detecting the activation of the polypeptide;
(3) detecting the interaction between the polypeptide and G-protein;
(4) detecting the activation of phospholipase C or its down stream pathway;
30 (5) detecting the activation of protein kinase cascade leading to activation of several kinases including Raf, MEK, ERKs, and protein kinase D (PKD);
(6) detecting the activation of a member of Src/Tec/Bmx-family of tyrosine kinases;
(7) detecting the activation of a member of the Ras and Rho family, regulation of a member of the JNK members of MAP families, or the reorganization of the actin cytoskeleton;

(8) detecting the activation of any signal complex mediated by the polypeptide activation;

(9) detecting the change in subcellular localization of the polypeptide including the ligand-induced internalization/endocytosis of the polypeptide;

5 (10) detecting the activation of any transcription factor downstream of the polypeptides or the activation of their downstream gene; and

(11) detecting cell proliferation, transformation, or any other oncogenic phenotype.

23. A method of treating or preventing NSCLC in a subject comprising administering to said subject an antisense composition, said composition comprising a nucleotide sequence complementary to a coding sequence of a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1.

10 24. A method of treating or preventing NSCLC in a subject comprising administering to said subject an siRNA composition comprising an siRNA, wherein said composition reduces the expression of a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1.

15 25. The method of claim 24, wherein the siRNA is a sense strand comprising the nucleotide sequence selected from the group of SEQ ID NOs: 32, 33, 34, 37, 39, and 40, as the target sequence.

20 26. The method of claim 25, wherein the siRNA has the general formula
S'-[A]-[B]-[A']-3'
wherein [A] is a ribonucleotide sequence corresponding to a sequence selected from the group consisting of SEQ ID NOs: 32, 33, 34, 37, 39, and 40; [B] is a ribonucleotide sequence consisting of 3 to 23 nucleotides; and [A'] is a ribonucleotide sequence complementary to [A].

25 27. A method for treating or preventing NSCLC in a subject comprising the step of administering to said subject a pharmaceutically effective amount of an antibody or fragment thereof that binds to a polypeptide encoded by a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1.

30 28. A method of treating or preventing NSCLC in a subject comprising administering to said subject a vaccine comprising a polypeptide encoded by a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1 or an immunologically active fragment of said polypeptide, or a polynucleotide encoding the polypeptide.

29. A method for treating or preventing NSCLC in a subject, said method comprising the step of administering a compound that is obtained by the method according to any one of claim 13

to claim 22.

30. A double-stranded molecule comprising a sense strand and an antisense strand, wherein the sense strand comprises a ribonucleotide sequence corresponding to a KIF11, GHSR1b, or NTSR1 target sequence, and wherein the antisense strand comprises a ribonucleotide sequence which is complementary to said sense strand, wherein said sense strand and said antisense strand hybridize to each other to form said double-stranded molecule, and wherein said double-stranded molecule, when introduced into a cell expressing a KIF11, GHSR1b, or NTSR1 gene, inhibits expression of said gene.
- 5 31. The double-stranded molecule of claim 30, wherein said KIF11, GHSR1b, or NTSR1 target sequence comprises at least about 10 contiguous nucleotides from the nucleotide sequences selected from the group of SEQ ID NOs: 1, 3 and 5.
- 10 32. The double-stranded molecule of claim 31, wherein said KIF11, GHSR1b, or NTSR1 target sequence comprises from about 19 to about 25 contiguous nucleotides from the nucleotide sequences selected from the group of SEQ ID NOs: 1, 3 and 5.
- 15 33. The double-stranded molecule of claim 32, wherein said KIF11, GHSR1b, or NTSR1 target sequence is selected from the group consisting of SEQ ID NOs: 32, 33, 34, 37, 39, and 40.
34. The double-stranded molecule of claim 30, wherein said double-stranded molecule is a single ribonucleotide transcript comprising the sense strand and the antisense strand linked via a single-stranded ribonucleotide sequence.
- 20 35. The double-stranded molecule of claim 30, wherein the double-stranded molecule is an oligonucleotide of less than about 100 nucleotides in length.
36. The double-stranded molecule of claim 35, wherein the double-stranded molecule is an oligonucleotide of less than about 75 nucleotides in length.
- 25 37. The double-stranded molecule of claim 36, wherein the double-stranded molecule is an oligonucleotide of less than about 50 nucleotides in length.
38. The double-stranded molecule of claim 37, wherein the double-stranded molecule is an oligonucleotide of less than about 25 nucleotides in length.
39. The double-stranded polynucleotide of claim 38, wherein the double stranded molecule is an oligonucleotide of between about 19 and about 25 nucleotides in length.
- 30 40. A vector encoding the double-stranded molecule of claim 30.

41. The vector of claim 40, wherein the vector encodes a transcript having a secondary structure and comprises the sense strand and the antisense strand.
42. The vector of claim 41, wherein the transcript further comprises a single-stranded ribonucleotide sequence linking said sense strand and said antisense strand.
- 5 43. A vector comprising a polynucleotide comprising a combination of a sense strand nucleic acid and an antisense strand nucleic acid, wherein said sense strand nucleic acid comprises nucleotide sequence selected from the group consisting of SEQ ID NOs: 32, 33, 34, 37, 39, and 40, and said antisense strand nucleic acid consists of a sequence complementary to the sense strand.
- 10 44 The vector of claim 43, wherein said polynucleotide has the general formula 5'-[A]-[B]-[A']-3'
wherein [A] is a nucleotide sequence selected from the group consisting of SEQ ID NOs: 32, 33, 34, 37, 39, and 40; [B] is a nucleotide sequence consisting of 3 to 23 nucleotides; and [A'] is a nucleotide sequence complementary to [A].
- 15 45. A composition for treating or preventing NSCLC, said composition comprising a pharmaceutically effective amount of an antisense polynucleotide against a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1.
46. A composition for treating or preventing NSCLC, said composition comprising a pharmaceutically effective amount of an siRNA against a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1.
- 20 25 47. The composition of claim 46, wherein the siRNA comprises a sense strand comprising the nucleotide sequence of SEQ ID NO: 32 to SEQ ID NO: 40, as the target sequence.
48. A composition for treating or preventing NSCLC, said composition comprising a pharmaceutically effective amount of an antibody or fragment thereof that binds to a polypeptide encoded by a gene selected from the group consisting of KIF11, GHSR1b, and NTSR1.
49. A composition for treating or preventing NSCLC, said composition comprising a pharmaceutically effective amount of the compound selected by the method of any one of claim 13 to claim 22 as an active ingredient, and a pharmaceutically acceptable carrier.

ABSTRACT

Disclosed are methods for detecting non-small cell lung cancer (NSCLC) using differentially expressed genes KIF11, GHSR1b, and NTSR1. Also disclosed are methods of identifying 5 compounds for treating and preventing NSCLC, based on the interaction between KOC1 and KIF11, or NMU and GHSR1b or NTSR1.

1 / 1 1

Fig. 1

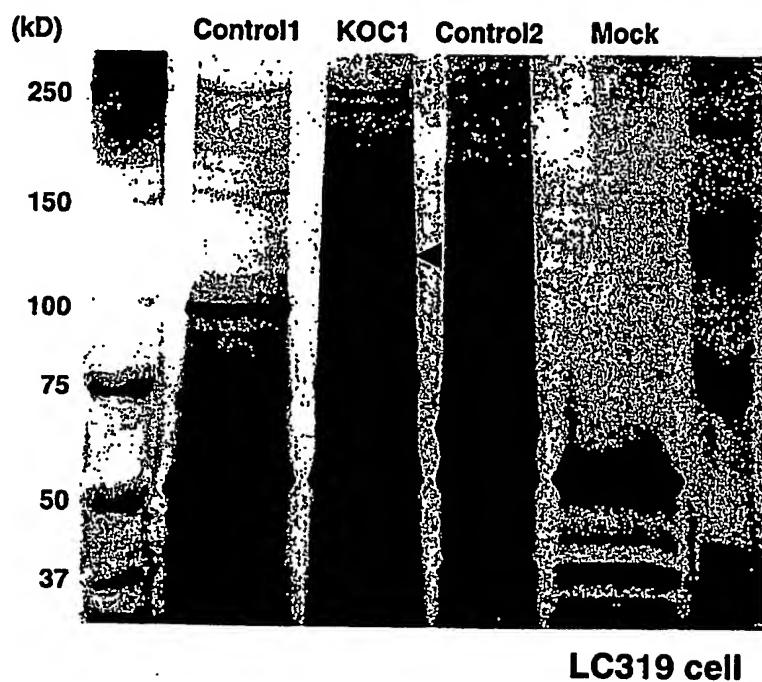
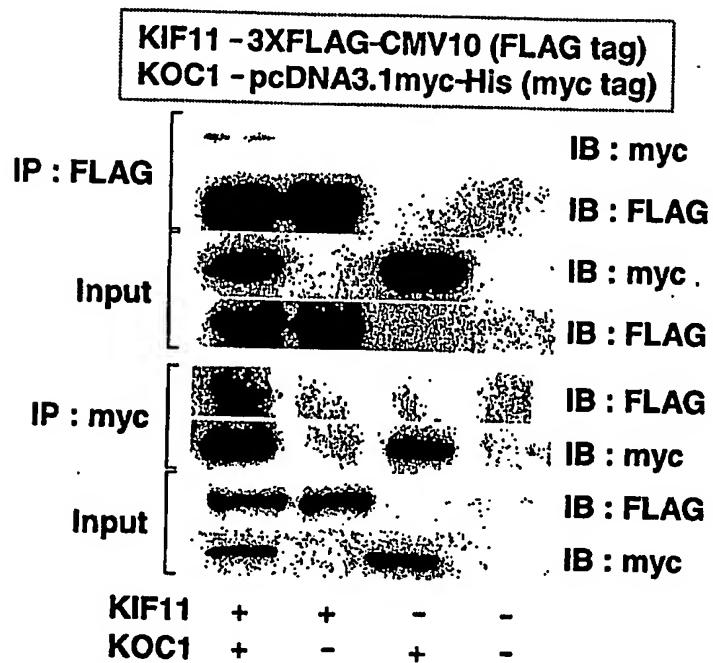


Fig. 2

a



b

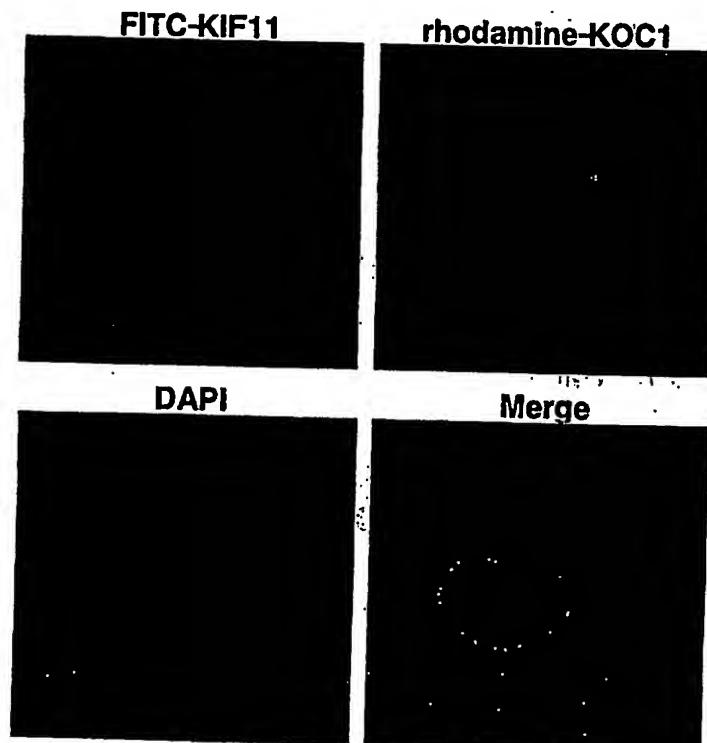
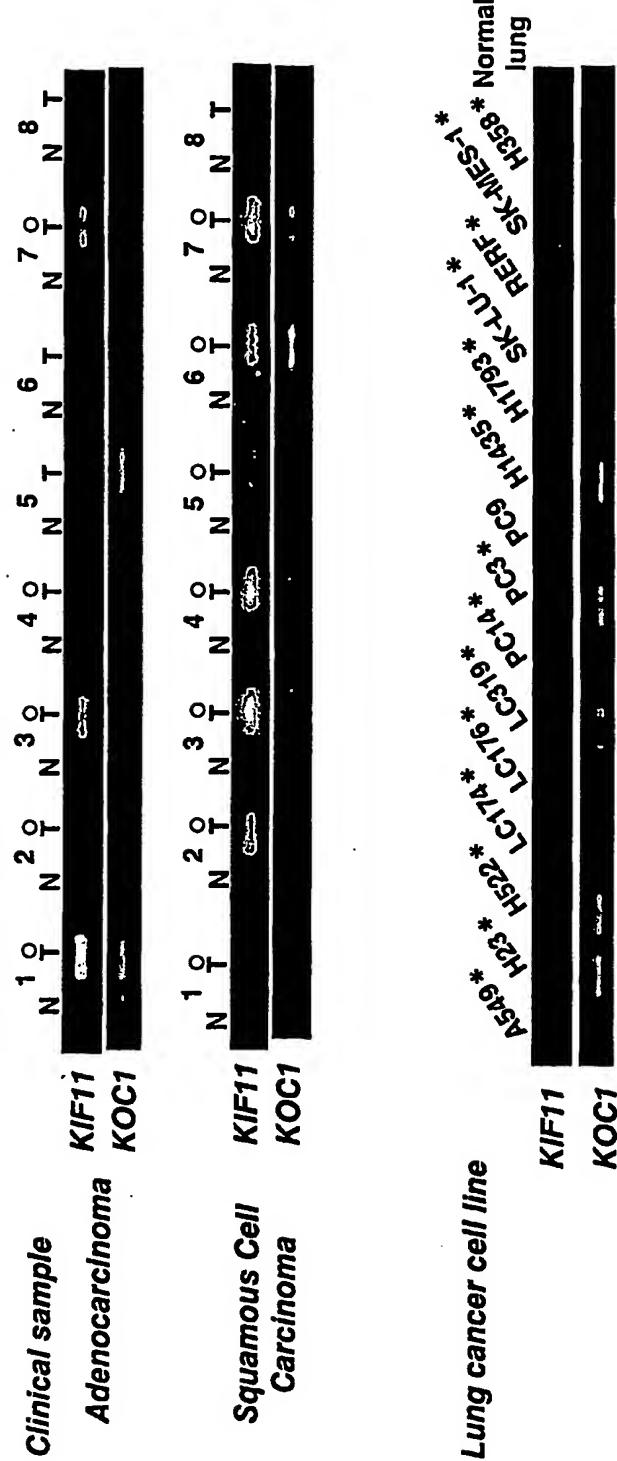


Fig. 3



4/11

Fig. 4

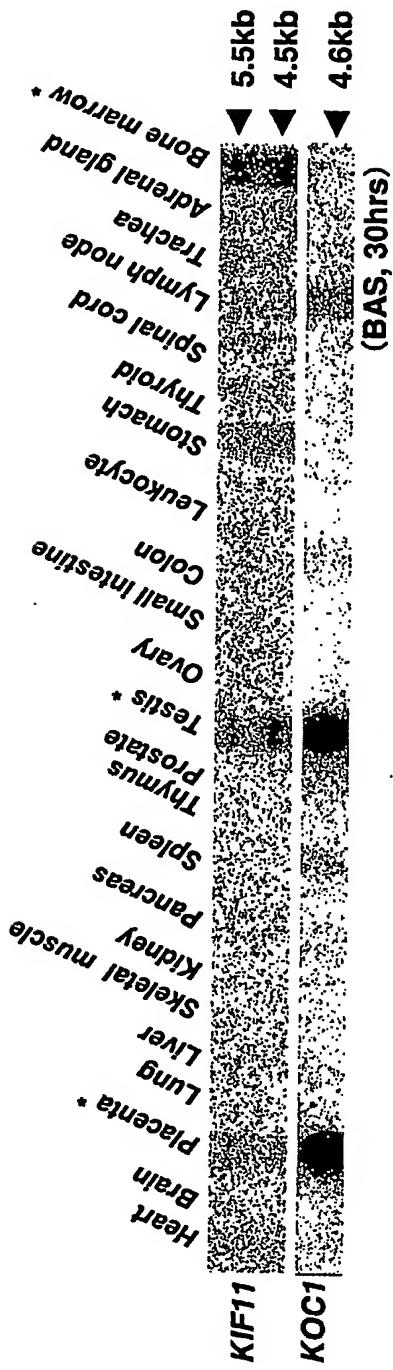
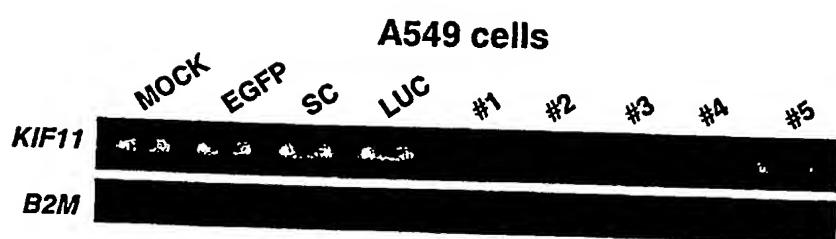


Fig. 5

5 / 1 1

a



b

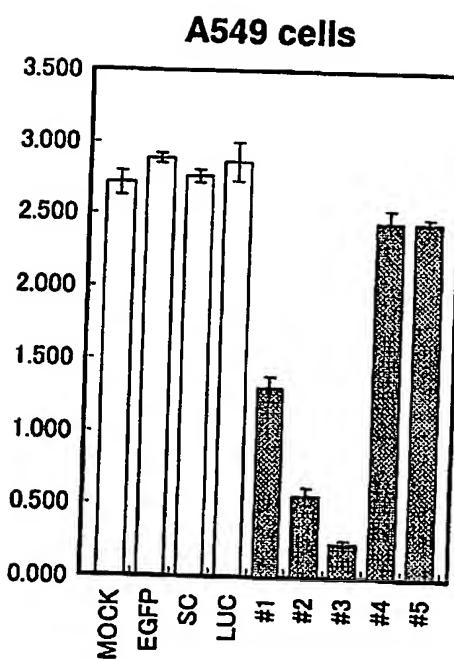


Fig. 6

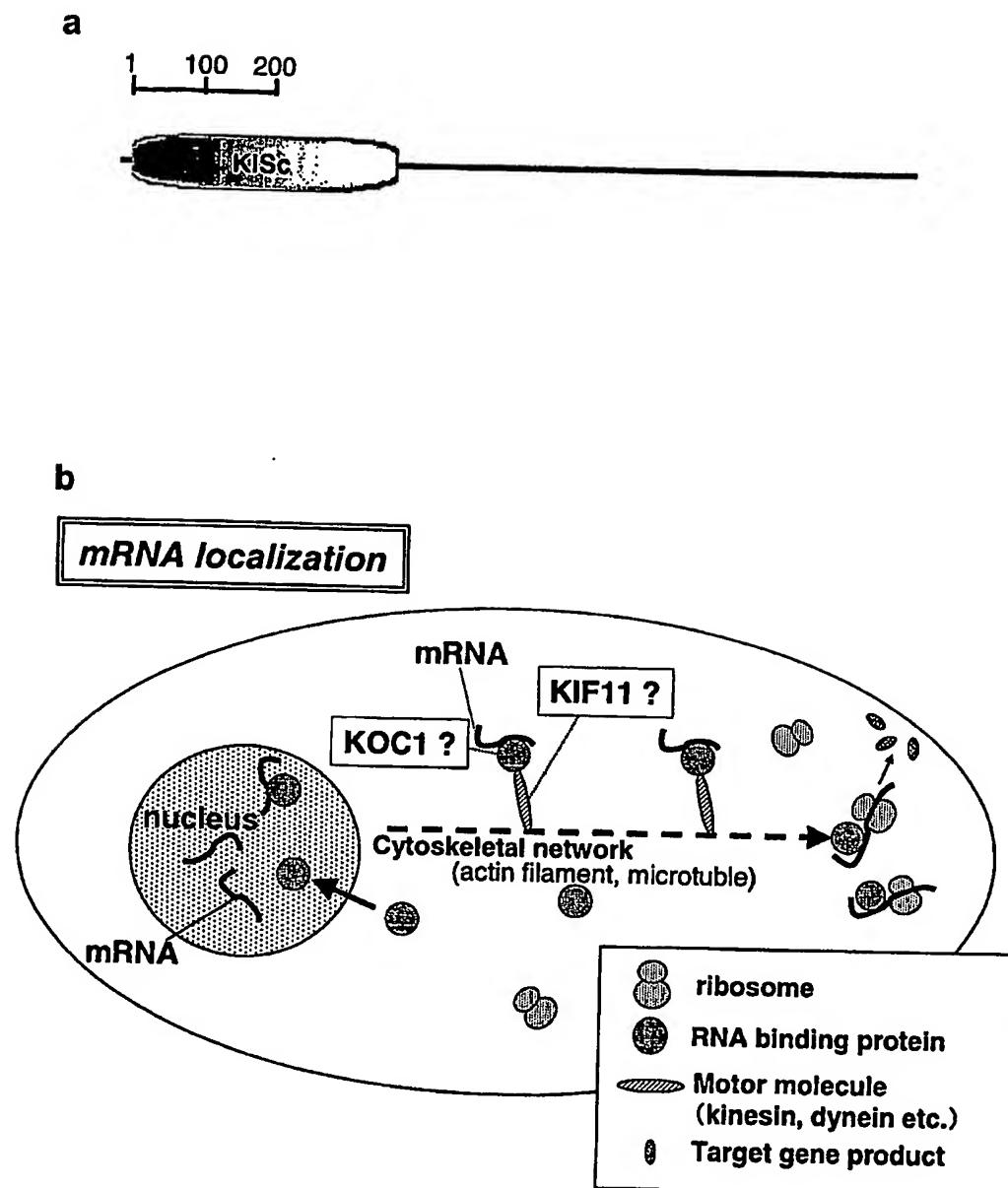


Fig. 7

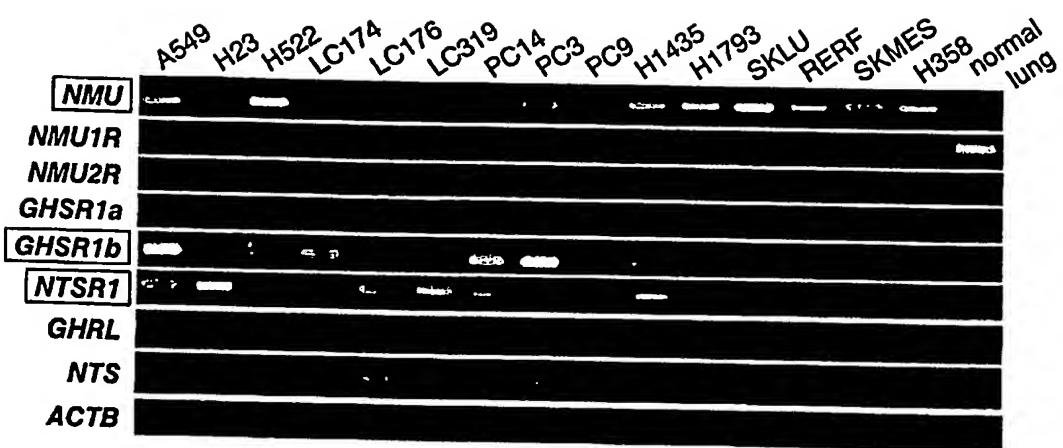
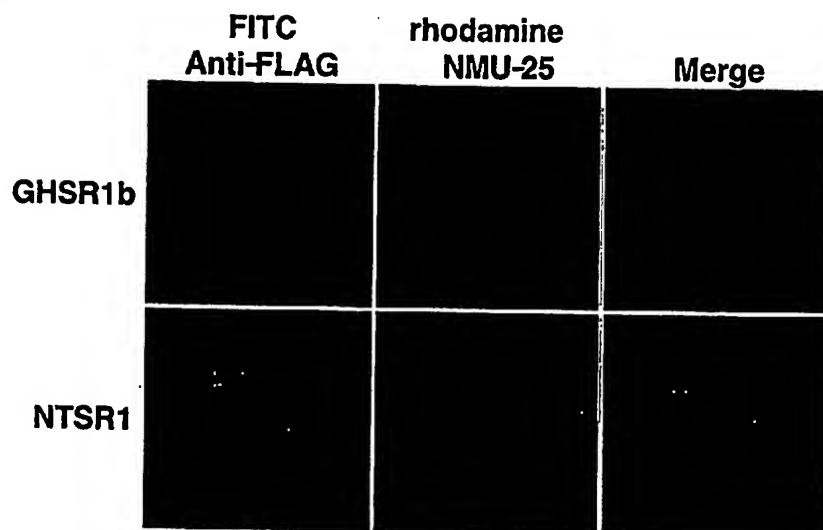
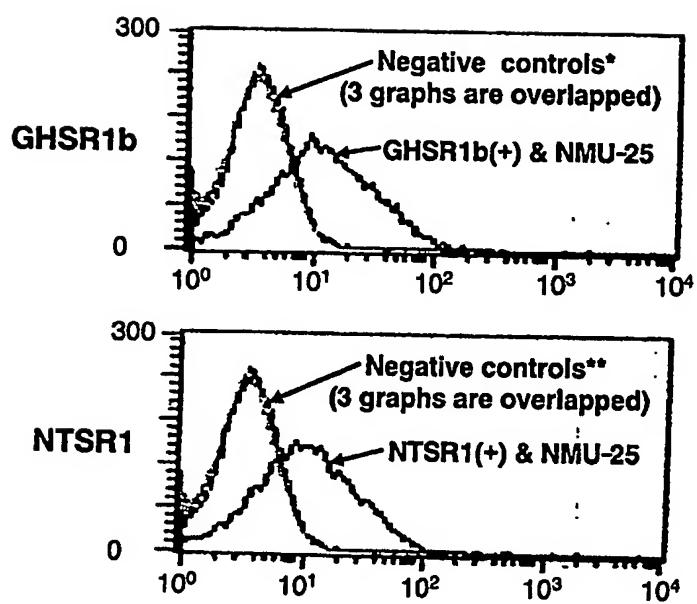


Fig. 8

a



b



*Three control assays used for this study:

1. COS-7 cells (no receptor) only
2. No receptor & NMU-25
3. GHSR1b(+)COS-7 cells only

**Three control assays used for this study:

1. COS-7 cells (no receptor) only
2. No receptor & NMU-25
3. NTSR1(+)COS-7 cells only

Fig. 9

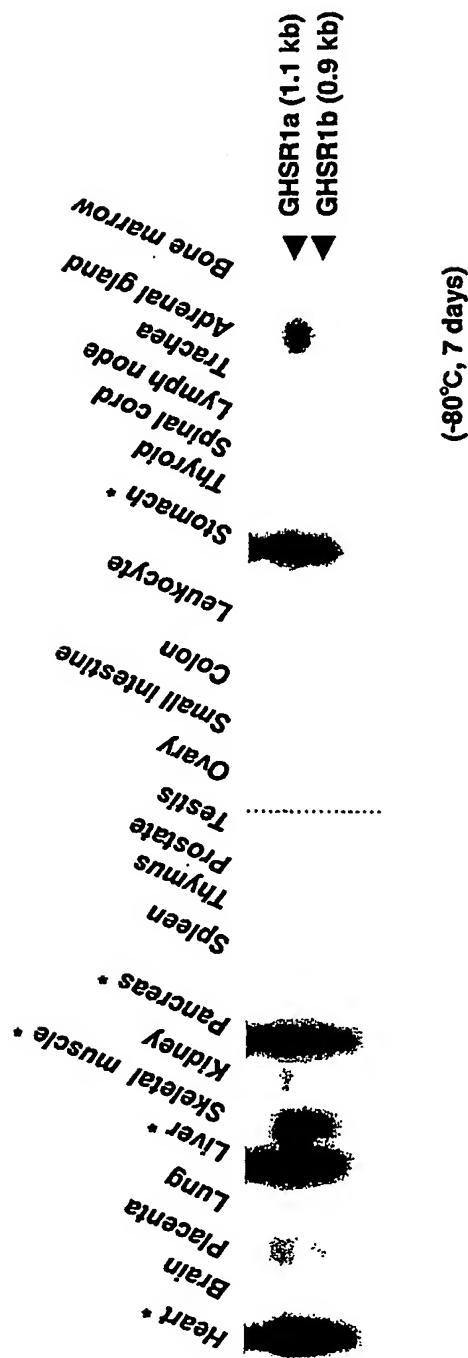
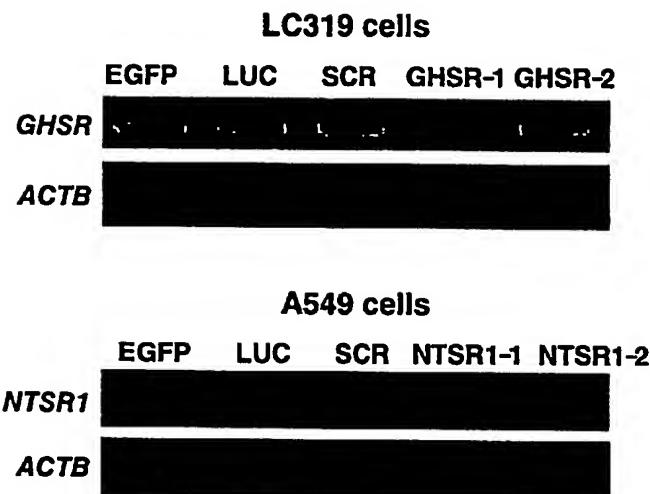
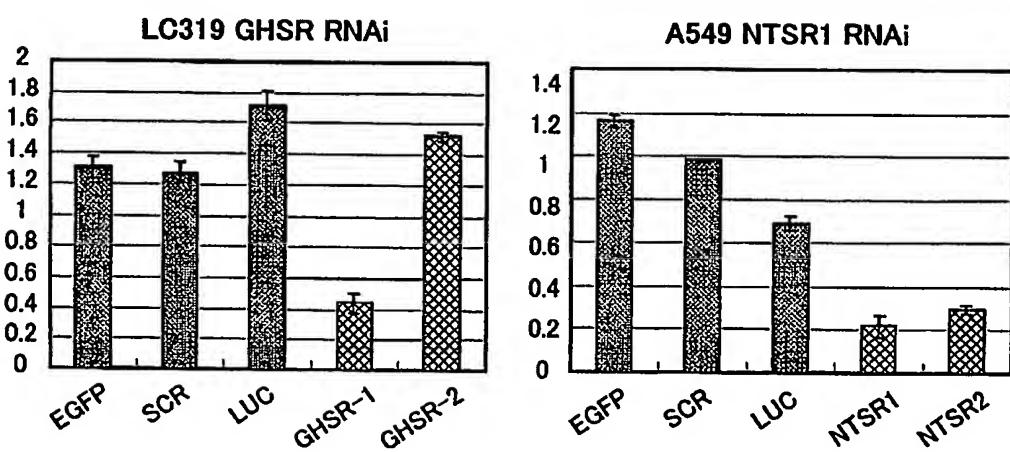


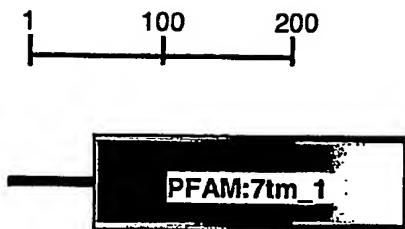
Fig. 10

a**b****MTT assay**

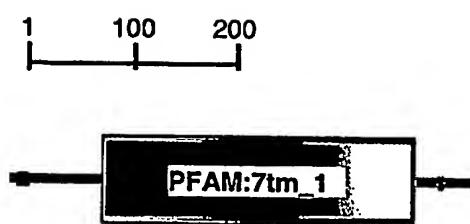
11/11

Fig. 11

a



b



SEQUENCE LISTING

<110> Nakamura, Yusuke
 Daigo, Yataro
 Nakatsuru, Shuichi

<120> METHOD FOR DIAGNOSING NON-SMALL CELL LUNG CANCER

<130> ONC-PRV0401/US

<160> 80

<170> Patentin version 3.1

<210> 1

<211> 4908

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (141)..(331)

<223>

<400> 1

acctgcgtgc agtcggtcct ccaggccacg cagcgccoga gagtaccagg gagactccgg 60
 cccctgtcgg cgccaaagccc ctccggccct cacagcgccc aggtccgcgg ccgggccttg 120
 attttttggc ggggaccgtc atg gcg tcg cag cca aat tcg tct gcg aag aag 173
 Met Ala Ser Gin Pro Asn Ser Ser Ala Lys Lys
 1 5 10

aaa gag gag aag ggg aag aac atc cag gtg gtg aga tgc aga cca 221
 Lys Glu Glu Lys Gly Lys Asn Ile Gin Val Val Val Arg Cys Arg Pro
 15 20 25

ttt aat ttg gca gag cgg aaa gct agc gcc cat tca ata gta gaa tgt 269
 Phe Asn Leu Ala Glu Arg Lys Ala Ser Ala His Ser Ile Val Glu Cys
 30 35 40

gat cct gta cga aaa gaa gtt agt gta cga act gga gga ttg gct gac 317
 Asp Pro Val Arg Lys Glu Val Ser Val Arg Thr Gly Gly Leu Ala Asp
 45 50 55

aag agc tca agg aaa aca tac act ttt gat atg gtg ttt gga gca tct 365
 Lys Ser Ser Arg Lys Thr Tyr Thr Phe Asp Met Val Phe Gly Ala Ser
 60 65 70

act aaa cag att gat gtt tac cga agt gtt gtt tgt cca att ctg gat 413
 Thr Lys Gin Ile Asp Val Tyr Arg Ser Val Val Cys Pro Ile Leu Asp
 80 85 90

gaa gtt att atg ggc tat aat tgc act atc ttt gcg tat ggc caa act 461
 Glu Val Ile Met Gly Tyr Asn Cys Thr Ile Phe Ala Tyr Gly Gln Thr
 95 100 105

ggc act gga aaa act ttt aca atg gaa ggt gaa agg tca cct aat gaa 509
 Gly Thr Gly Lys Thr Phe Thr Met Glu Gly Glu Arg Ser Pro Asn Glu
 110 115 120

gag tat acc tgg gaa gag gat ccc ttg gct ggt ata att cca cgt acc Glu Tyr Thr Trp Glu Glu Asp Pro Leu Ala Gly Ile Ile Pro Arg Thr 125 130 135	557
ctt cat caa att ttt gag aaa ctt act gat aat ggt act gaa ttt tca Leu His Gln Ile Phe Glu Lys Leu Thr Asp Asn Gly Thr Glu Phe Ser 140 145 150 155	605
gtc aaa gtg tct ctg ttg gag atc tat aat gaa gag ctt ttt gat ctt Val Lys Val Ser Leu Leu Glu Ile Tyr Asn Glu Glu Leu Phe Asp Leu 160 165 170	653
ctt aat cca tca tot gat gtt tct gag aga cta cag atg ttt gat gat Leu Asn Pro Ser Ser Asp Val Ser Glu Arg Leu Gln Met Phe Asp Asp 175 180 185	701
ccc cgt aac aag aga gga gtg ata att aaa ggt tta gaa gaa att aca Pro Arg Asn Lys Arg Gly Val Ile Ile Lys Gly Leu Glu Glu Ile Thr 190 195 200	749
gta cac aac aag gat gaa gtc tat caa att tta gaa aag ggg gca gca Val His Asn Lys Asp Glu Val Tyr Gln Ile Leu Glu Lys Gly Ala Ala 205 210 215	797
aaa agg aca act gca gct act ctg atg aat gca tac tct agt cgt tcc Lys Arg Thr Thr Ala Ala Thr Leu Met Asn Ala Tyr Ser Ser Arg Ser 220 225 230 235	845
cac tca gtt ttc tct gtt aca ata cat atg aaa gaa act acg att gat His Ser Val Phe Ser Val Thr Ile His Met Lys Glu Thr Thr Ile Asp 240 245 250	893
gga gaa gag ctt gtt aaa atc gga aag ttg aac ttg gtt gat ctt gca Gly Glu Glu Leu Val Lys Ile Gly Lys Leu Asn Leu Val Asp Leu Ala 255 260 265	941
gga agt gaa aac att ggc cgt tct gga gct gtt gat aag aga gct cgg Gly Ser Glu Asn Ile Gly Arg Ser Gly Ala Val Asp Lys Arg Ala Arg 270 275 280	989
gaa gct gga aat ata aat caa tcc ctg ttg act ttg gga agg gtc att Glu Ala Gly Asn Ile Asn Gln Ser Leu Leu Thr Leu Gly Arg Val Ile 285 290 295	1037
act gcc ctt gta gaa aga aca cct cat gtt cot tat cga gaa tct aaa Thr Ala Leu Val Glu Arg Thr Pro His Val Pro Tyr Arg Glu Ser Lys 300 305 310 315	1085
cta act aga atc ctc cag gat tct ctt gga ggg cgt aca aga aca tot Leu Thr Arg Ile Leu Gln Asp Ser Leu Gly Gly Arg Thr Arg Thr Ser 320 325 330	1133
ata att gca aca att tct cct gca tct ctc aat ctt gag gaa act ctg Ile Ile Ala Thr Ile Ser Pro Ala Ser Leu Asn Leu Glu Glu Thr Leu 335 340 345	1181
agt aca ttg gaa tat gct cat aga gca aag aac ata ttg aat aag cct Ser Thr Leu Glu Tyr Ala His Arg Ala Lys Asn Ile Leu Asn Lys Pro 350 355 360	1229

gaa gtg aat cag aaa ctc acc aaa aaa gct ctt att aag gag tat acg Glu Val Asn Gln Lys Leu Thr Lys Lys Ala Leu Ile Lys Glu Tyr Thr 365 370 375	1277
gag gag ata gaa cgt tta aaa cga gat ctt gct gca gcc cgt gag aaa Glu Glu Ile Glu Arg Leu Lys Arg Asp Leu Ala Ala Arg Glu Lys 380 385 390 395	1325
aat gga gtg tat att tct gaa gaa aat ttt aga gtc atg agt gga aaa Asn Gly Val Tyr Ile Ser Glu Glu Asn Phe Arg Val Met Ser Gly Lys 400 405 410	1373
tta act gtt caa gaa gag cag att gta gaa ttg att gaa aaa att egt Leu Thr Val Gln Glu Glu Gln Ile Val Glu Leu Ile Glu Lys Ile Gly 415 420 425	1421
gct gtt gag gag gag ctg aat agg gtt aca gag ttg ttt atg gat aat Ala Val Glu Glu Leu Asn Arg Val Thr Glu Leu Phe Met Asp Asn 430 435 440	1469
aaa aat gaa ctt gac cag tgt aaa tct gac ctg caa aat aaa aca caa Lys Asn Glu Leu Asp Gln Cys Lys Ser Asp Leu Gln Asn Lys Thr Gln 445 450 455	1517
gaa ctt gaa acc act caa aaa cat ttg caa gaa act aaa tta caa ctt Glu Leu Glu Thr Thr Gln Lys His Leu Gln Glu Thr Lys Leu Gln Leu 460 465 470 475	1565
gtt aaa gaa gaa tat atc aca tca gct ttg gaa agt act gag gag aaa Val Lys Glu Glu Tyr Ile Thr Ser Ala Leu Glu Ser Thr Glu Glu Lys 480 485 490	1613
ctt cat gat gct gcc agc aag ctg ctt aac aca gtt gaa gaa act aca Leu His Asp Ala Ala Ser Lys Leu Leu Asn Thr Val Glu Glu Thr Thr 495 500 505	1661
aaa gat gta tot ggt ctc cat tcc aaa ctg gat cgt aag aag gca gtt Lys Asp Val Ser Gly Leu His Ser Lys Leu Asp Arg Lys Lys Ala Val 510 515 520	1709
gac caa cac aat gca gaa gct cag gat att ttt ggc aaa aac ctg aat Asp Gln His Asn Ala Glu Ala Gln Asp Ile Phe Gly Lys Asn Leu Asn 525 530 535	1757
agt ctg ttt aat aat atg gaa gaa tta att aag gat ggc agc tca aag Ser Leu Phe Asn Asn Met Glu Glu Leu Ile Lys Asp Gly Ser Ser Lys 540 545 550 555	1805
caa aag gcc atg cta gaa gta cat aag acc tta ttt ggt aat ctg ctg Gln Lys Ala Met Leu Glu Val His Lys Thr Leu Phe Gly Asn Leu Leu 560 565 570	1853
tct tcc agt gtc tct gca tta gat acc att act aca gta gca ctt gga Ser Ser Ser Val Ser Ala Leu Asp Thr Ile Thr Thr Val Ala Leu Gly 575 580 585	1901
tct ctc aca tct att cca gaa aat gtg tct act cat gtt tct cag att Ser Leu Thr Ser Ile Pro Glu Asn Val Ser Thr His Val Ser Gln Ile 590 595 600	1949

ttt aat atg ata cta aaa gaa caa tca tta gca gca gaa agt aaa act Phe Asn Met Ile Leu Lys Glu Gin Ser Leu Ala Ala Glu Ser Lys Thr 605 610 615	1997
gta cta cag gaa ttg att aat gta ctc aag act gat ctt cta agt tca Val Leu Gin Glu Leu Ile Asn Val Leu Lys Thr Asp Leu Leu Ser Ser 620 625 630 635	2045
ctg gaa atg att tta tcc cca act gtg gtg tct ata ctg aaa atc aat Leu Glu Met Ile Leu Ser Pro Thr Val Val Ser Ile Leu Lys Ile Asn 640 645 650	2093
agt caa cta aag cat att ttc aag act tca ttg aca gtg gcc gat aag Ser Gin Leu Lys His Ile Phe Lys Thr Ser Leu Thr Val Ala Asp Lys 655 660 665	2141
ata gaa gat caa aaa aag gaa cta gat ggo ttt ctc agt ata ctg tgt Ile Glu Asp Gin Lys Glu Leu Asp Gly Phe Leu Ser Ile Leu Cys 670 675 680	2189
aac aat cta cat gaa cta caa gaa aat acc att tgt tcc ttg gtt gag Asn Asn Leu His Glu Leu Gin Glu Asn Thr Ile Cys Ser Leu Val Glu 685 690 695	2237
tca caa aag caa tgt gga aac cta act gaa gac ctg aag aca ata aag Ser Gin Lys Gin Cys Gly Asn Leu Thr Glu Asp Leu Lys Thr Ile Lys 700 705 710 715	2285
cag acc cat tcc cag gaa ctt tgc aag tta atg aat ctt tgg aca gag Gin Thr His Ser Gin Glu Leu Cys Lys Leu Met Asn Leu Trp Thr Glu 720 725 730	2333
aga ttc tgt gct ttg gag gaa aag tgt gaa aat ata cag aaa cca ctt Arg Phe Cys Ala Leu Glu Glu Lys Cys Glu Asn Ile Gin Lys Pro Leu 735 740 745	2381
agt agt gtc cag gaa aat ata cag cag aaa tot aag gat ata gtc aac Ser Ser Val Gin Glu Asn Ile Gin Gin Lys Ser Lys Asp Ile Val Asn 750 755 760	2429
aaa atg act ttt cac agt caa aaa ttt tgt gct gat tot gat ggc ttc Lys Met Thr Phe His Ser Gin Lys Phe Cys Ala Asp Ser Asp Gly Phe 765 770 775	2477
tca cag gaa ctc aga aat ttt aac caa gaa ggt aca aaa ttg gtt gaa Ser Gin Glu Leu Arg Asn Phe Asn Gin Glu Gly Thr Lys Leu Val Glu 780 785 790 795	2525
gaa tot gtg aaa cac tot gat aaa ctc aat ggc aac ctg gaa aaa ata Glu Ser Val Lys His Ser Asp Lys Leu Asn Gly Asn Leu Glu Lys Ile 800 805 810	2573
tct caa gag act gaa cag aga tgt gaa tct ctg aac aca aga aca gtt Ser Gin Glu Thr Glu Gin Arg Cys Glu Ser Leu Asn Thr Arg Thr Val 815 820 825	2621
tat ttt tct gaa cag tgg gta tot tcc tta aat gaa agg gaa cag gaa Tyr Phe Ser Glu Gin Trp Val Ser Ser Leu Asn Glu Arg Glu Gin Glu 830 835 840	2669

ONC-PRV0401SQ.txt

ctt cac aac tta ttg gag gtt gta agc caa tgt tgt gag gct tca agt 2717
 Leu His Asn Leu Leu Glu Val Val Ser Gin Cys Cys Glu Ala Ser Ser
 845 850 855
 tca gac atc act gag aaa tca gat gga cgt aag gca gct cat gag aaa 2765
 Ser Asp Ile Thr Glu Lys Ser Asp Gly Arg Lys Ala Ala His Glu Lys
 860 865 870 875
 cag cat aac att ttt ott gat cag atg act att gat gaa gat aaa ttg 2813
 Gin His Asn Ile Phe Leu Asp Gin Met Thr Ile Asp Glu Asp Lys Leu
 880 885 890
 ata gca caa aat cta gaa ctt aat gaa acc ata aaa att ggt ttg act 2861
 Ile Ala Gin Asn Leu Glu Leu Asn Glu Thr Ile Lys Ile Gly Leu Thr
 895 900 905
 aag ctt aat tgc ttt ctg gaa cag gat ctg aaa ctg gat atc cca aca 2909
 Lys Leu Asn Cys Phe Leu Glu Gin Asp Leu Lys Leu Asp Ile Pro Thr
 910 915 920
 ggt acg aca cca cag agg aaa agt tat tta tac cca tca aca ctg gta 2957
 Gly Thr Thr Pro Gin Arg Lys Ser Tyr Leu Tyr Pro Ser Thr Leu Val
 925 930 935
 aga act gaa cca cgt gaa cat ctc ctt gat cag ctg aaa agg aaa cag 3005
 Arg Thr Glu Pro Arg Glu His Leu Leu Asp Gin Leu Lys Arg Lys Gin
 940 945 950 955
 oct gag ctg tta atg atg cta aac tgt tca gaa aac aac aaa gaa gag 3053
 Pro Glu Leu Leu Met Met Leu Asn Cys Ser Glu Asn Asn Lys Glu Glu
 960 965 970
 aca att ccg gat gtg gat gta gaa gag gca gtt ctg ggg cag tat act 3101
 Thr Ile Pro Asp Val Asp Val Glu Glu Ala Val Leu Gly Gin Tyr Thr
 975 980 985
 gaa gaa cct cta agt caa gag cca tct gta gat gct ggt gtg gat tgt 3149
 Glu Glu Pro Leu Ser Gin Glu Pro Ser Val Asp Ala Gly Val Asp Cys
 990 995 1000
 tca tca att ggo ggg gtt cca ttt ttc cag cat aaa aaa tca cat 3194
 Ser Ser Ile Gly Gly Val Pro Phe Phe Gin His Lys Lys Ser His
 1005 1010 1015
 gga aaa gac aaa gaa aac aga ggc att aac aca ctg gag agg tct 3239
 Gly Lys Asp Lys Glu Asn Arg Gly Ile Asn Thr Leu Glu Arg Ser
 1020 1025 1030
 aaa gtg gaa gaa act aca gag cac ttg gtt aca aag agc aga tta 3284
 Lys Val Glu Glu Thr Thr Glu His Leu Val Thr Lys Ser Arg Leu
 1035 1040 1045
 cct ctg cga gcc cag atc aac ctt taa ttcaattggg gtttggcaat 3331
 Pro Leu Arg Ala Gin Ile Asn Leu
 1050 1055
 tttatTTTA aagaaaaactt aaaaaataaaa cctgaaacc cagaacttga gccttgtgtta 3391
 tagatTTTA aagaatataat atatcagcog ggccgggtgg ctcatgcgt taatcccagc 3451
 acitTTGGGAG gctgaggcgg gtggattgt tgagcccagg agtttgagac cagcgtggcc 3511

aacgtggcaa aacctcgct ctgttaaaaa	ttagccggc	gtgggtggcac	actccctgtaa	3571	
tcccgactac tggggaggct gaggcacgag	aatcaacttga	accaggaaag	cgggggttgc	3631	
gtgagccaaa ggtacaccac tacactccag	cctggcaac	agagacaagac	tccggctcaaa	3691	
aaacaaaatt taaaaagat ataaggcagt	actgttaaatt	cagttttaatt	ttgatatact	3751	
cccattttc tgcacccct atagttact	ttgttattaaa	ttgggttca	tttgggattt	3811	
gcaatgtaaa tacgtatttc tagtttcat	ataaaagtagt	tottttataa	caaataaaaa	3871	
gtattttct tgcataattat	taagtaatga	atataaaga	actgtactct	3931	
agcttaacat aggtttatata	caccaacatc	tgcctttaga	aaggaccatc	3991	
tttcttgc	tgactttgtt	attttcttgc	atccctccct	4051	
tcctcgctc acttttcc	tttttatttt	toaccaaaacc	attttagag	ctacaaaacc	4111
tatccttct tattttcagt	agtccagaatt	ttatctagaa	atcttttaac	acctttttag	4171
tgttttttc taaaatcaact	gtcaacaata	aatctaacc	tagttttatc	cctccctttaa	4231
gtatttaaaa ctgttgc	caaatgtgaa	agcatttaat	tcctttaaga	ggccctaaactc	4291
attcacccgt	acaggttca	aaaaaggccc	acttttaggt	atacattgt	4351
accaccaga	catctgacta	atggctctgt	gcoacactcc	aagaccgttg	4411
aagctcacaa	tgattttaagg	actgtttgaa	acttccaaatt	atgtctataa	4471
tttggttaca	tgatgaaact	tttggttgtt	gcttgggtt	atataataca	4531
gtatctttt	ctcgattcaa	atcttaaccc	ttaggactct	gttattttt	4591
catatttctg	gaagttgaga	tgtttcagct	tgaagaacca	aaacagaagg	4651
aagaataaaat	tttctgctca	cgatgagttt	agtgtgtaaa	gtttagagac	4711
gatagctaaa	ttaaaccaaa	ccctattgaa	gaattgaata	tatgctaott	4771
aattgatctc	gtagaatttat	cttataaaaa	taatggctat	aatttctctg	4831
tgtcagcata	agogatggat	aataccta	aaactgcct	cagtaaatcc	4891
aatgtgttt	ctacatt				4908

<210> 2

<211> 1056

<212> PRT

<213> Homo sapiens

<400> 2

Met	Ala	Ser	Gln	Pro	Asn	Ser	Ser	Ala	Lys	Lys	Lys	Glu	Glu	Lys	Gly
1								5		10				15	

Lys	Asn	Ile	Gln	Val	Val	Val	Arg	Cys	Arg	Pro	Phe	Asn	Leu	Ala	Glu
								20		25			30		

Arg	Lys	Ala	Ser	Ala	His	Ser	Ile	Val	Glu	Cys	Asp	Pro	Val	Arg	Lys
							35		40			45			

Glu	Val	Ser	Val	Arg	Thr	Gly	Gly	Leu	Ala	Asp	Lys	Ser	Ser	Arg	Lys
						50		55			60				

Thr	Tyr	Thr	Phe	Asp	Met	Val	Phe	Gly	Ala	Ser	Thr	Lys	Gln	Ile	Asp
						65		70			75			80	

Val	Tyr	Arg	Ser	Val	Val	Cys	Pro	Ile	Leu	Asp	Glu	Val	Ile	Met	Gly
						85		90			95				

Tyr	Asn	Cys	Thr	Ile	Phe	Ala	Tyr	Gly	Gln	Thr	Gly	Thr	Gly	Lys	Thr
						100		105			110				

Phe	Thr	Met	Glu	Gly	Glu	Arg	Ser	Pro	Asn	Glu	Glu	Tyr	Thr	Trp	Glu
						115		120			125				

Glu	Asp	Pro	Leu	Ala	Gly	Ile	Ile	Pro	Arg	Thr	Leu	His	Gln	Ile	Phe
						130		135			140				

Glu	Lys	Leu	Thr	Asp	Asn	Gly	Thr	Glu	Phe	Ser	Val	Lys	Val	Ser	Leu
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

145	150	155	160
Leu Glu Ile Tyr Asn Glu Glu Leu Phe Asp Leu Leu Asn Pro Ser Ser			
165	170	175	
Asp Val Ser Glu Arg Leu Gin Met Phe Asp Asp Pro Arg Asn Lys Arg			
180	185	190	
Gly Val Ile Ile Lys Gly Leu Glu Glu Ile Thr Val His Asn Lys Asp			
195	200	205	
Glu Val Tyr Gin Ile Leu Glu Lys Gly Ala Ala Lys Arg Thr Thr Ala			
210	215	220	
Ala Thr Leu Met Asn Ala Tyr Ser Ser Arg Ser His Ser Val Phe Ser			
225	230	235	240
Val Thr Ile His Met Lys Glu Thr Thr Ile Asp Gly Glu Glu Leu Val			
245	250	255	
Lys Ile Gly Lys Leu Asn Leu Val Asp Leu Ala Gly Ser Glu Asn Ile			
260	265	270	
Gly Arg Ser Gly Ala Val Asp Lys Arg Ala Arg Glu Ala Gly Asn Ile			
275	280	285	
Asn Gin Ser Leu Leu Thr Leu Gly Arg Val Ile Thr Ala Leu Val Glu			
290	295	300	
Arg Thr Pro His Val Pro Tyr Arg Glu Ser Lys Leu Thr Arg Ile Leu			
305	310	315	320
Gin Asp Ser Leu Gly Gly Arg Thr Arg Thr Ser Ile Ile Ala Thr Ile			
325	330	335	
Ser Pro Ala Ser Leu Asn Leu Glu Glu Thr Leu Ser Thr Leu Glu Tyr			
340	345	350	
Ala His Arg Ala Lys Asn Ile Leu Asn Lys Pro Glu Val Asn Gin Lys			
355	360	365	
Leu Thr Lys Ala Leu Ile Lys Glu Tyr Thr Glu Glu Ile Glu Arg			
370	375	380	
Leu Lys Arg Asp Leu Ala Ala Ala Arg Glu Lys Asn Gly Val Tyr Ile			
385	390	395	400
Ser Glu Glu Asn Phe Arg Val Met Ser Gly Lys Leu Thr Val Gin Glu			
405	410	415	
Glu Gin Ile Val Glu Leu Ile Glu Lys Ile Gly Ala Val Glu Glu Glu			
420	425	430	
Leu Asn Arg Val Thr Glu Leu Phe Met Asp Asn Lys Asn Glu Leu Asp			
435	440	445	
Gin Cys Lys Ser Asp Leu Gin Asn Lys Thr Gin Glu Leu Glu Thr Thr			
450	455	460	
Gin Lys His Leu Gin Glu Thr Lys Leu Gin Leu Val Lys Glu Glu Tyr			

465	470	475	480
Ile Thr Ser Ala Leu Glu Ser Thr Glu Glu Lys Leu His Asp Ala Ala			
485	490	495	
Ser Lys Leu Leu Asn Thr Val Glu Glu Thr Thr Lys Asp Val Ser Gly			
500	505	510	
Leu His Ser Lys Leu Asp Arg Lys Lys Ala Val Asp Gln His Asn Ala			
515	520	525	
Glu Ala Gln Asp Ile Phe Gly Lys Asn Leu Asn Ser Leu Phe Asn Asn			
530	535	540	
Met Glu Glu Leu Ile Lys Asp Gly Ser Ser Lys Gln Lys Ala Met Leu			
545	550	555	560
Glu Val His Lys Thr Leu Phe Gly Asn Leu Leu Ser Ser Ser Val Ser			
565	570	575	
Ala Leu Asp Thr Ile Thr Thr Val Ala Leu Gly Ser Leu Thr Ser Ile			
580	585	590	
Pro Glu Asn Val Ser Thr His Val Ser Gln Ile Phe Asn Met Ile Leu			
595	600	605	
Lys Glu Gln Ser Leu Ala Ala Glu Ser Lys Thr Val Leu Gln Glu Leu			
610	615	620	
Ile Asn Val Leu Lys Thr Asp Leu Leu Ser Ser Leu Glu Met Ile Leu			
625	630	635	640
Ser Pro Thr Val Val Ser Ile Leu Lys Ile Asn Ser Gln Leu Lys His			
645	650	655	
Ile Phe Lys Thr Ser Leu Thr Val Ala Asp Lys Ile Glu Asp Gln Lys			
660	665	670	
Lys Glu Leu Asp Gly Phe Leu Ser Ile Leu Cys Asn Asn Leu His Glu			
675	680	685	
Leu Gln Glu Asn Thr Ile Cys Ser Leu Val Glu Ser Gln Lys Gln Cys			
690	695	700	
Gly Asn Leu Thr Glu Asp Leu Lys Thr Ile Lys Gln Thr His Ser Gln			
705	710	715	720
Glu Leu Cys Lys Leu Met Asn Leu Trp Thr Glu Arg Phe Cys Ala Leu			
725	730	735	
Glu Glu Lys Cys Glu Asn Ile Gln Lys Pro Leu Ser Ser Val Gln Glu			
740	745	750	
Asn Ile Gln Gln Lys Ser Lys Asp Ile Val Asn Lys Met Thr Phe His			
755	760	765	
Ser Gln Lys Phe Cys Ala Asp Ser Asp Gly Phe Ser Gln Glu Leu Arg			
770	775	780	
Asn Phe Asn Gln Glu Gly Thr Lys Leu Val Glu Glu Ser Val Lys His			

ONC-PRV0401SQ.txt

785 790 795 800
Ser Asp Lys Leu Asn Gly Asn Leu Glu Lys Ile Ser Gin Glu Thr Glu
805 810 815
Gln Arg Cys Glu Ser Leu Asn Thr Arg Thr Val Tyr Phe Ser Glu Gln
820 825 830
Trp Val Ser Ser Leu Asn Glu Arg Glu Gln Glu Leu His Asn Leu Leu
835 840 845
Glu Val Val Ser Gin Cys Cys Glu Ala Ser Ser Asp Ile Thr Glu
850 855 860
Lys Ser Asp Gly Arg Lys Ala Ala His Glu Lys Gln His Asn Ile Phe
865 870 880
Leu Asp Gin Met Thr Ile Asp Glu Asp Lys Leu Ile Ala Gln Asn Leu
885 890 895
Glu Leu Asn Glu Thr Ile Lys Ile Gly Leu Thr Lys Leu Asn Cys Phe
900 905 910
Leu Glu Gin Asp Leu Lys Leu Asp Ile Pro Thr Gly Thr Thr Pro Gin
915 920 925
Arg Lys Ser Tyr Leu Tyr Pro Ser Thr Leu Val Arg Thr Glu Pro Arg
930 935 940
Glu His Leu Leu Asp Gin Leu Lys Arg Lys Gln Pro Glu Leu Leu Met
945 950 960
Met Leu Asn Cys Ser Gin Asn Asn Lys Glu Glu Thr Ile Pro Asp Val
965 970 975
Asp Val Glu Glu Ala Val Leu Gly Gin Tyr Thr Glu Glu Pro Leu Ser
980 985 990
Gin Glu Pro Ser Val Asp Ala Gly Val Asp Cys Ser Ser Ile Gly Gly
995 1000 1005
Val Pro Phe Phe Gin His Lys Lys Ser His Gly Lys Asp Lys Glu
1010 1015 1020
Asn Arg Gly Ile Asn Thr Leu Glu Arg Ser Lys Val Glu Glu Thr
1025 1030 1035
Thr Glu His Leu Val Thr Lys Ser Arg Leu Pro Leu Arg Ala Gin
1040 1045 1050
Ile Asn Leu
1055

<210> 3
<211> 870
<212> DNA
<213> Homo sapiens
<220>

<221> CDS
 <222> (1).. (870)
 <223>

 <400> 3
 atg tgg aac gcg acg ccc ago gaa gag cog ggg ttc aac ctc aca ctg 48
 Met Trp Asn Ala Thr Pro Ser Glu Glu Pro Gly Phe Asn Leu Thr Leu
 1 5 10 15

 gcc gac ctg gac tgg gat gct tcc ccc ggc aac gac tcg ctg ggc gac 96
 Ala Asp Leu Asp Trp Asp Ala Ser Pro Gly Asn Asp Ser Leu Gly Asp
 20 25 30

 gag ctg ctg cag ctc ttc ccc gcg cog ctg ctg ggc gtc aca gcc 144
 Glu Leu Leu Gln Leu Phe Pro Ala Pro Leu Leu Ala Gly Val Thr Ala
 35 40 45

 aco tgc gtg gca ctc ttc gtg gtg ggt atc gct ggc aac ctg ctc acc 192
 Thr Cys Val Ala Leu Phe Val Val Gly Ile Ala Gly Asn Leu Leu Thr
 50 55 60

 atg ctg gtg gtg tgg cgc ttc cgc gag ctg cgc acc acc acc aac ctc 240
 Met Leu Val Val Ser Arg Phe Arg Glu Leu Arg Thr Thr Thr Asn Leu
 65 70 75 80

 tac ctg tcc agc atg gcc ttc tcc gat ctg ctc atc ttc ctc tgc atg 288
 Tyr Leu Ser Ser Met Ala Phe Ser Asp Leu Leu Ile Phe Leu Cys Met
 85 90 95

 ccc ctg gac ctc gtt cgc ctc tgg cag tac cgg ccc tgg aac ttc ggc 336
 Pro Leu Asp Leu Val Arg Leu Trp Gln Tyr Arg Pro Trp Asn Phe Gly
 100 105 110

 gac ctc ctc tgc aaa ctc ttc caa ttc gtc agt gag agc tgc acc tac 384
 Asp Leu Leu Cys Lys Leu Phe Gln Phe Val Ser Glu Ser Cys Thr Tyr
 115 120 125

 gcc acg gtg ctc acc ato aca gcg ctg agc gtc gag cgc tac ttc gcc 432
 Ala Thr Val Leu Thr Ile Thr Ala Leu Ser Val Glu Arg Tyr Phe Ala
 130 135 140

 atc tgc ttc cca ctc cgg gcc aag gtg gtg gtc acc aag ggg cgg gtg 480
 Ile Cys Phe Pro Leu Arg Ala Lys Val Val Thr Lys Gly Arg Val
 145 150 155 160

 aag ctg gtc atc ttc gtc atc tgg gcc gtg gcc ttc tgc ago gcc ggg 528
 Lys Leu Val Ile Phe Val Ile Trp Ala Val Ala Phe Cys Ser Ala Gly
 165 170 175

 ccc atc ttc gtg cta gtc ggg gtg gag cac gag aac ggc acc gac cct 576
 Pro Ile Phe Val Leu Val Gly Val Glu His Glu Asn Gly Thr Asp Pro
 180 185 190

 tgg gac acc aac gag tgc cgc ccc acc gag ttt gcg gtg cgc tct gga 624
 Trp Asp Thr Asn Glu Cys Arg Pro Thr Glu Phe Ala Val Arg Ser Gly
 195 200 205

 ctg ctc acg gtc atg gtg tgg gtg toc agc atc ttc ttc ttc ctt cct 672
 Leu Leu Thr Val Met Val Trp Val Ser Ser Ile Phe Phe Phe Leu Pro
 210 215 220

gtc ttc tgt ctc acg gtc ctc tac agt ctc atc ggc agg aag ctg tgg Val Phe Cys Leu Thr Val Leu Tyr Ser Leu Ile Gly Arg Lys Leu Trp 225 230 235 240	720
cgg agg agg cgc ggc gat got gtc gtg ggt gco tcg ctc agg gac cag Arg Arg Arg Arg Gly Asp Ala Val Val Gly Ala Ser Leu Arg Asp Gln 245 250 255	768
aac cac aag caa acc gtg aaa atg ctg ggt ggg tct cag cgc gcg ctc Asn His Lys Gln Thr Val Lys Met Leu Gly Gly Ser Gln Arg Ala Leu 260 265 270	816
agg ctt tct ctc gcg ggt cct atc ctc ctg tgc ott ctc cct tct Arg Leu Ser Leu Ala Gly Pro Ile Leu Ser Leu Cys Leu Leu Pro Ser 275 280 285	864
ctc tga Leu	870

<210> 4

<211> 289

<212> PRT

<213> Homo sapiens

<400> 4

Met Trp Asn Ala Thr Pro Ser Glu Glu Pro Gly Phe Asn Leu Thr Leu 1 5 10 15

Ala Asp Leu Asp Trp Asp Ala Ser Pro Gly Asn Asp Ser Leu Gly Asp 20 25 30

Glu Leu Leu Gln Leu Phe Pro Ala Pro Leu Leu Ala Gly Val Thr Ala 35 40 45

Thr Cys Val Ala Leu Phe Val Val Gly Ile Ala Gly Asn Leu Leu Thr 50 55 60

Met Leu Val Val Ser Arg Phe Arg Glu Leu Arg Thr Thr Thr Asn Leu 65 70 75 80

Tyr Leu Ser Ser Met Ala Phe Ser Asp Leu Leu Ile Phe Leu Cys Met 85 90 95

Pro Leu Asp Leu Val Arg Leu Trp Gln Tyr Arg Pro Trp Asn Phe Gly 100 105 110

Asp Leu Leu Cys Lys Leu Phe Gln Phe Val Ser Glu Ser Cys Thr Tyr 115 120 125

Ala Thr Val Leu Thr Ile Thr Ala Leu Ser Val Glu Arg Tyr Phe Ala 130 135 140

Ile Cys Phe Pro Leu Arg Ala Lys Val Val Val Thr Lys Gly Arg Val 145 150 155 160

Lys Leu Val Ile Phe Val Ile Trp Ala Val Ala Phe Cys Ser Ala Gly 165 170 175

Pro Ile Phe Val Leu Val Gly Val Glu His Glu Asn Gly Thr Asp Pro
 180 185 190
 Trp Asp Thr Asn Glu Cys Arg Pro Thr Glu Phe Ala Val Arg Ser Gly
 195 200 205
 Leu Leu Thr Val Met Val Trp Val Ser Ser Ile Phe Phe Leu Pro
 210 215 220
 Val Phe Cys Leu Thr Val Leu Tyr Ser Leu Ile Gly Arg Lys Leu Trp
 225 230 235 240
 Arg Arg Arg Arg Gly Asp Ala Val Val Gly Ala Ser Leu Arg Asp Gln
 245 250 255
 Asn His Lys Gln Thr Val Lys Met Leu Gly Gly Ser Gln Arg Ala Leu
 260 265 270
 Arg Leu Ser Leu Ala Gly Pro Ile Leu Ser Leu Cys Leu Leu Pro Ser
 275 280 285
 Leu

<210> 5
 <211> 4131
 <212> DNA
 <213> Homo sapiens

 <220>
 <221> CDS
 <222> (373)..(1629)
 <223>

 <400> 5
 tcaagctcgc cccgcgcagc ccgagccggg ctggcgctg tccctggggg cctggggaa 60
 cgcgcgttt ggagatcgg a ggcacctgg a acccggtggca agcgccgagc cgggagacag 120
 cccgaggAAC caccgggttct ggagcttagga gccggaagct gggagtccgg aggagagcgg 180
 agcccgagc cccgagcccg gggggggcgg tctgggtctg ggcgttcccg actggacggc 240
 ggcggccgtg gtcttcggca cgcgcocctcc cctgggtctg cgttcatcgg tcccccctg 300
 agacgcgcgc actccctggcc gggacttccag ccccgagggc gcccggacaga gcccggggact 360

 ccagcgccca cc atg cgc ctc aac agc tcc gcg ccg gga acc ccg ggc acg 411
 Met Arg Leu Asn Ser Ser Ala Pro Gly Thr Pro Gly Thr
 1 5 10

 ccg gcc gac ccc ttc cag cgg gcg cag gcc gga otg gag gag gcg 459
 Pro Ala Ala Asp Pro Phe Gln Arg Ala Gln Ala Gly Leu Glu Ala
 15 20 25

 ctg ctg gcc ccg ggc ttc ggc aac gct tcg ggc aac gcg tcg gag gag cgc 507
 Leu Leu Ala Pro Gly Phe Gly Asn Ala Ser Gly Asn Ala Ser Glu Arg
 30 35 40 45

 gtc ctg gcg gca ccc agc agc gag ctg gac gtg aac acc gac atc tac 555
 Val Leu Ala Ala Pro Ser Ser Glu Leu Asp Val Asn Thr Asp Ile Tyr
 50 55 60

tcc aaa gtg ctg gtg acc gcc gtg tac ctg gcg ctc ttc gtg gtg ggc Ser Lys Val Leu Val Thr Ala Val Tyr Leu Ala Leu Phe Val Val Gly 65 70 75	603
acg gtg ggc aac acg gtg acg gcg ttc acg ctg gcg cgg aag aag tcg Thr Val Gly Asn Thr Val Ala Phe Thr Leu Ala Arg Lys Lys Ser 80 85 90	651
ctg cag agc ctg cag agc acg gtg cat tac cac ctg ggc ago ctg gcg Leu Gln Ser Leu Gln Ser Thr Val His Tyr His Leu Gly Ser Leu Ala 95 100 105	699
ctg tcc gac ctg ctc acc ctg ctg gcc atg ccc gtg gag ctg tac Leu Ser Asp Leu Leu Thr Leu Leu Ala Met Pro Val Glu Leu Tyr 110 115 120 125	747
aac ttc atc tgg gtg cac cac ccc tgg gcc ttc ggc gac gcc ggc tgc Asn Phe Ile Trp Val His His Pro Trp Ala Phe Gly Asp Ala Gly Cys 130 135 140	795
cgc ggc tao tac ttc ctg cgc gac gcc tgc acc tac gcc acg gcc ctc Arg Gly Tyr Tyr Phe Leu Arg Asp Ala Cys Thr Tyr Ala Thr Ala Leu 145 150 155	843
aac gtg gcc ago ctg agt gtg gag cgc tac ctg gcc atc tgc cac ccc Asn Val Ala Ser Leu Ser Val Glu Arg Tyr Leu Ala Ile Cys His Pro 160 165 170	891
ttc aag gcc aag acc ctc atg tcc cga agc cgc acc aag aag ttc atc Phe Lys Ala Lys Thr Leu Met Ser Arg Ser Arg Thr Lys Lys Phe Ile 175 180 185	939
agc gcc atc tgg ctc gcc tcc ctg ctg acg gtg cct atg ctg ttc Ser Ala Ile Trp Leu Ala Ser Ala Leu Leu Thr Val Pro Met Leu Phe 190 195 200 205	987
acc atg ggc gag cag aac cgc agc gcc gac ggc cag cac gcc ggc ggc Thr Met Gly Glu Gln Asn Arg Ser Ala Asp Gly Gln His Ala Gly Gly 210 215 220	1035
ctg gtg tgc acc ccc acc atc cac act gcc acc gtc aag gtc gtc ata Leu Val Cys Thr Pro Thr Ile His Thr Ala Thr Val Lys Val Val Ile 225 230 235	1083
cag gtc aac acc ttc atg tcc ttc ata ttc ccc atg gtg gtc atc tog Gln Val Asn Thr Phe Met Ser Phe Ile Phe Pro Met Val Val Ile Ser 240 245 250	1131
gtc ctg aac acc atc atc gcc aac aag ctg aac gtc atg gta cgc cag Val Leu Asn Thr Ile Ile Ala Asn Lys Leu Thr Val Met Val Arg Gln 255 260 265	1179
gcg gcc gag cag ggc caa gtg tgc acg gtc ggg ggc gag cac agc aca Ala Ala Glu Gln Gly Gln Val Cys Thr Val Gly Gly Glu His Ser Thr 270 275 280 285	1227
ttc agc atg gcc ato gag cct ggc agg gtc cag gcc ctg cgg cac ggc Phe Ser Met Ala Ile Glu Pro Gly Arg Val Gln Ala Leu Arg His Gly 290 295 300	1275

ONC-PRV0401SQ.txt

gtg cgc gtc cta cgt gca gtg gtc atc gcc ttt gtg gtc tgc tgg ctg Val Arg Val Leu Arg Ala Val Val Ile Ala Phe Val Val Cys Trp Leu 305 310 315	1323
ccc tac cac gtg ogg cgc ctc atg ttc tgc tac atc atc tcg gat gag cag Pro Tyr His Val Arg Arg Leu Met Phe Cys Tyr Ile Ser Asp Glu Gln 320 325 330	1371
tgg act ccc ttc ctc tat gac ttc tac cac tac ttc tac atg gtg acc Trp Thr Pro Phe Leu Tyr Asp Phe Tyr His Tyr Phe Tyr Met Val Thr 335 340 345	1419
aac gca ctc ttc tac gtc agc tcc acc atc aac ccc atc ctg tac aac Asn Ala Leu Phe Tyr Val Ser Ser Thr Ile Asn Pro Ile Leu Tyr Asn 350 355 360 365	1467
ctc gtc tct gcc aac ttc cgc cac atc ttc ctg gcc aca ctg gcc tgc Leu Val Ser Ala Asn Phe Arg His Ile Phe Leu Ala Thr Leu Ala Cys 370 375 380	1515
ctc tgc ccg gtg tgg cgg cgc agg agg aag agg cca gcc ttc tcg agg Leu Cys Pro Val Trp Arg Arg Arg Lys Arg Pro Ala Phe Ser Arg 385 390 395	1563
aag gcc gac agc gtg tcc agc aac cac acc ctc tcc agc aat gcc acc Lys Ala Asp Ser Val Ser Ser Asn His Thr Leu Ser Ser Asn Ala Thr 400 405 410	1611
cgc gag acg ctg tac tag gtcgtgcgcc cccggaaacgtg tccaggagga Arg Glu Thr Leu Tyr 415	1659
gcctggccat gggccttgc ccccgacaga cagacgaccc cccacccggg agccttgatg ggggtcaggc agaggccagc ctgcactgga gtctgaggcc tgggacccccc ccctccacc ccctaaccctat ttttttcat tagtgttcccccggggctgtc cccaaactct ccccacccct cccccatoto ctctttgaaa gccagaacaa gagagcgtc ctotccca tagaaaaagg gcctctaaca aggaaaaatt agtgtgcggc aaaaggcagt tttctttgtt ctcagactaa tggatgggtc cagagaagga aatgaaatgt gtcgggtggg gccgggccto cggcggcccg gtctgttccatgttccac atctctgagg cctgcacccc ctctgtctag ctccgggagt ccagccccag tcccgcaggc tccgtggcgtt tgggctcac gtgcagaccc tgcacatgcag acccatggcc ccctccccca ggcagotcca agaaagctcc ctgactcgcc ctttcaggcc tggcaagctg gggggccatc gccgtgggaa gtccctccca ccacccctgc cgcaggcagc tgcagcccccc agaggggacc acaagcccaa aaaggacaaa aatgggctgg ctggaaatgg cccagacccc agcctccctt ctcctccatc atccatcaccc aggccaaggc ccaggggctc tgccaggaca ccacatggga gggggctcag gtcctcagcct caagatcttc agctgtggcc tctcgggctc ggcagaaggc agccggatc agggggctgg totccagcac ctccgggat ggccgtggcc agatgggtt ggcattcccg tgcattttgc tttagtctgt gcaggctgag gtctggagcc aggccccagag ctggcttccag gttggggctc tgagaaggaa aatgggac agggggcgtatgtc tctgagtaag atggccaggc ccaggaactc aggtttcagg tgagaaggag cgggtgttcc aggcaccgtc ggccggcagc cttgggttga ggcacagact catttgcac cttctggccg cggcagccct ggccccggcc tccaaagcgt tgaaaaagct ggccgttccat tggctcttag gatccaggct ccacagagca catgacttagc caggccctg gtttaagaag gtgcgttcaag ctcataaggaaa gagatccca ggagaaggctg gcccggacca ggcaggagct gggagccaca ggaaggcaaaa gtcagccctt tottcaaggg atttccctgt ctcagaggcag ctttgccttcc agggaaatgg gtcgttggct ggctgcctgc accggccatg tgcacccagg accccggacac ctggcttccag gtcgttccat gccacttgc ctctctggaa ctcaggttcc oogtcgttcaag aatggagatc gaatgttaca gtatgtcag tggcttggat ctggcttggc agttgacggg ttcccttgcac cccacaaaat ccctctccaa ccacaggacc cttcggctca ccaagaacgg gggccagggg agtcaggccctt attcgttgcac tttctgtccca aactttggcc ccaacaacgc ggtcatcagc caggcagccc tcccagtgcc caaggccac 3339	1719 1779 1839 1899 1959 2019 2079 2139 2199 2259 2319 2379 2439 2499 2559 2619 2679 2739 2799 2859 2919 2979 3039 3099 3159 3219 3279 3339

caaccccagg	gaaacaggc	cagcacagag	gggccttct	cccccacaga	gctcccattga	3399
catagtctgc	tctggcgga	agagcttgc	tgccagccag	ggatgtccag	agtgcggtgc	3459
agccctatac	cctgctcagg	agtgggctca	gagttagca	aatgctaagg	cccttcaggc	3519
tgggctctga	acgaggacct	ggactcagag	ccagacaggg	cagcctcaga	cccttcctgc	3579
gggtctctgg	accttggco	ataatttctg	agcctcggtt	tcccatcta	agaacacat	3639
gtggtcgttc	cgccctctca	gctggatgag	actgtctgg	aggatccacc	ccggaaacaga	3699
cagaacggtg	tcttcagga	tggtgctctg	agagaggcga	gagtggatgc	cccaactgccc	3759
tagaccctcg	gtagacgtgg	gttctctggg	ggggggctcg	tggotgtgac	tgaagtgogg	3819
tttcccccttg	atgttttgat	gctcttatct	gtgcacttac	cgttagttagg	gacacgtgtc	3879
catgcaccac	agacacacacc	acgacacactg	atctcgatc	actagttgc	ggccagggtca	3939
tgatgtggcc	cggaaagctg	ccctcgctg	ccatgagtgc	gtcggctatg	gagtccggag	3999
ccctgagcc	ggccctgtgt	gacggcacag	ccctcacacg	tcaaacgccc	accccaactc	4059
ccaccatctg	cagggtgtga	aaacaaaccc	cgtgtatctc	tcaataaagg	tggccgaagg	4119
gcctcgatgt	gg					4131

<210> 6

<211> 418

<212> PRT

<213> Homo sapiens

<400> 6

Met	Arg	Leu	Asn	Ser	Ser	Ala	Pro	Gly	Thr	Pro	Gly	Thr	Pro	Ala	Ala
1						5			10			15			

Asp	Pro	Phe	Gln	Arg	Ala	Gln	Ala	Gly	Leu	Glu	Glu	Ala	Leu	Leu	Ala
	20						25					30			

Pro	Gly	Phe	Gly	Asn	Ala	Ser	Gly	Asn	Ala	Ser	Glu	Arg	Val	Leu	Ala
	35						40					45			

Ala	Pro	Ser	Ser	Glu	Leu	Asp	Val	Asn	Thr	Asp	Ile	Tyr	Ser	Lys	Val
	50					55					60				

Leu	Val	Thr	Ala	Val	Tyr	Leu	Ala	Leu	Phe	Val	Val	Gly	Thr	Val	Gly
	65					70					75				80

Asn	Thr	Val	Thr	Ala	Phe	Thr	Leu	Ala	Arg	Lys	Lys	Ser	Leu	Gln	Ser
	85						90					95			

Leu	Gln	Ser	Thr	Val	His	Tyr	His	Leu	Gly	Ser	Leu	Ala	Leu	Ser	Asp
	100						105					110			

Leu	Leu	Thr	Leu	Leu	Ala	Met	Pro	Val	Glu	Leu	Tyr	Asn	Phe	Ile
	115					120					125			

Trp	Val	His	His	Pro	Trp	Ala	Phe	Gly	Asp	Ala	Gly	Cys	Arg	Gly	Tyr
	130					135					140				

Tyr	Phe	Leu	Arg	Asp	Ala	Cys	Thr	Tyr	Ala	Thr	Ala	Leu	Asn	Val	Ala
	145					150					155				160

Ser	Leu	Ser	Val	Glu	Arg	Tyr	Leu	Ala	Ile	Cys	His	Pro	Phe	Lys	Ala
	165						170					175			

Lys	Thr	Leu	Met	Ser	Arg	Ser	Arg	Thr	Lys	Lys	Phe	Ile	Ser	Ala	Ile
	180						185					190			

Trp	Leu	Ala	Ser	Ala	Leu	Leu	Thr	Val	Pro	Met	Leu	Phe	Thr	Met	Gly
	195						200					205			

Glu Gln Asn Arg Ser Ala Asp Gly Gln His Ala Gly Gly Leu Val Cys
 210 215 220
 Thr Pro Thr Ile His Thr Ala Thr Val Lys Val Val Ile Gln Val Asn
 225 230 235 240
 Thr Phe Met Ser Phe Ile Phe Pro Met Val Val Ile Ser Val Leu Asn
 245 250 255
 Thr Ile Ile Ala Asn Lys Leu Thr Val Met Val Arg Gln Ala Ala Glu
 260 265 270
 Gln Gly Gln Val Cys Thr Val Gly Gly Glu His Ser Thr Phe Ser Met
 275 280 285
 Ala Ile Glu Pro Gly Arg Val Gln Ala Leu Arg His Gly Val Arg Val
 290 295 300
 Leu Arg Ala Val Val Ile Ala Phe Val Val Cys Trp Leu Pro Tyr His
 305 310 315 320
 Val Arg Arg Leu Met Phe Cys Tyr Ile Ser Asp Glu Gln Trp Thr Pro
 325 330 335
 Phe Leu Tyr Asp Phe Tyr His Tyr Phe Tyr Met Val Thr Asn Ala Leu
 340 345 350
 Phe Tyr Val Ser Ser Thr Ile Asn Pro Ile Leu Tyr Asn Leu Val Ser
 355 360 365
 Ala Asn Phe Arg His Ile Phe Leu Ala Thr Leu Ala Cys Leu Cys Pro
 370 375 380
 Val Trp Arg Arg Arg Lys Arg Pro Ala Phe Ser Arg Lys Ala Asp
 385 390 395 400
 Ser Val Ser Ser Asn His Thr Leu Ser Ser Asn Ala Thr Arg Glu Thr
 405 410 415
 Leu Tyr

<210> 7
 <211> 23
 <212> DNA
 <213> Artificial
 <220>
 <223> An artificially synthesized primer sequence for RT-PCR
 <400> 7
 taaatggctt caggagactt cag

23

<210> 8
 <211> 24
 <212> DNA
 <213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 8
ggttttaaat gcagctccata tgtg

24

<210> 9
<211> 23
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 9
ctgaacagtgcgtatcttcc tta

23

<210> 10
<211> 23
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 10
gatggcttctt gacttagagg ttc

23

<210> 11
<211> 22
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 11
tgaagagatt cagagtggac ga

22

<210> 12
<211> 23
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 12
actgagaaca ttgacaacac agg

23

<210> 13
<211> 22
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 13
aagaggggaca gggacaagta gt

22

<210> 14
<211> 21
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 14
atgccactgt tactgttca g

21

<210> 15
<211> 23
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 15
ggctcttaca actcatgtac cca

23

<210> 16
<211> 24
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 16
tgatacagag acatgaagtg agca

24

<210> 17
<211> 19
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 17
tggtgtttgc cttcatctt

19

<210> 18
<211> 20
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 18
gaatcccgaga agtctgaaca

20

<210> 19
<211> 19
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 19
acggtcctct acagtcata

19

<210> 20
<211> 18
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 20
cacaggagaga ggatagga

18

<210> 21
<211> 23
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 21
agtgggtca gagtotagca aat

23

<210> 22
<211> 23
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR

<400> 22
tattgagaga tacacgggtt ttg

23

<210> 23
<211> 21
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR
<400> 23
tgagccctga acaccagaga g

21

<210> 24
<211> 21
<212> DNA
<213> Artificial
<220>
<223> An artificially synthesized primer sequence for RT-PCR
<400> 24
aaagccagat gagcgttct a

21

<210> 25
<211> 22
<212> DNA
<213> Artificial
<220>
<223> An artificially synthesized primer sequence for RT-PCR
<400> 25
tcttcagcat gatgtgttgt gt

22

<210> 26
<211> 24
<212> DNA
<213> Artificial
<220>
<223> An artificially synthesized primer sequence for RT-PCR
<400> 26
tgagagattc atgaggaagt cttg

24

<210> 27
<211> 21
<212> DNA
<213> Artificial
<220>
<223> An artificially synthesized primer sequence for RT-PCR
<400> 27
gaggtgatag cattgttttc g

21

<210> 28
<211> 21
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR
<400> 28
caagtcatgt tacaggtaag c

21

<210> 29
<211> 19
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized target sequence for siRNA
<400> 29
gaagcagcac gacttcttc

19

<210> 30
<211> 19
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized target sequence for siRNA
<400> 30
cgtacgcgga atacttoga

19

<210> 31
<211> 19
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized target sequence for siRNA
<400> 31
gcgcgcgttg taggatcc

19

<210> 32
<211> 19
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized target sequence for siRNA
<400> 32
gttagtgtac gaactggag

19

<210> 33
<211> 19
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized target sequence for siRNA

<400> 33
gtgtctctgt tggagatct

19

<210> 34
<211> 19
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized target sequence for siRNA

<400> 34
gaaggcagtt gaccaacac

19

<210> 35
<211> 19
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized target sequence for siRNA

<400> 35
gagactgaac agagatgtg

19

<210> 36
<211> 19
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized target sequence for siRNA

<400> 36
gccaatgtt tgaggcttc

19

<210> 37
<211> 19
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized target sequence for siRNA

<400> 37
cctctacctg tccagcatg

19

<210> 38
<211> 19
<212> DNA
<213> Artificial

<220>

<223> An artificially synthesized target sequence for siRNA

<400> 38

gctggtcatc ttctgtcatc

19

<210> 39

<211> 19

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized target sequence for siRNA

<400> 39

gttcatcagc gccatctgg

19

<210> 40

<211> 19

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized target sequence for siRNA

<400> 40

ggtcgtcata caggtcaac

19

<210> 41

<211> 32

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 41

ggaattccat gtggAACGCG acgcccAGCG aa

32

<210> 42

<211> 40

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized primer sequence for RT-PCR

<400> 42

cgcggatccg cgtgtattaa tactagattc tgtccaggcc

40

<210> 43

<211> 32

<212> DNA

<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR
<400> 43
ggaattccat gtggAACGCG acGCCAGCG aa

32

<210> 44
<211> 36
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR
<400> 44
cgcggatcog cggagagaag ggagaaggca caggga

36

<210> 45
<211> 36
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for RT-PCR
<400> 45
ggaattccat ggcctcaac agtccgcgc cgggaa

36

<210> 46
<211> 39
<212> DNA
<213> Artificial.

<220>
<223> An artificially synthesized primer sequence for RT-PCR
<400> 46
cgcggatccg cggtagacgcg tctcgccgggt ggcattgtct

39

<210> 47
<211> 22
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized primer sequence for PCR of H1RNA gene promoter region.

<400> 47
tggtagccaa gtgcaggta ta

22

<210> 48
<211> 22
<212> DNA

<213> Artificial
<220>
<223> An artificially synthesized primer sequence for PCR of H1RNA gene promoter region.

<400> 48
ccaaagggtt tctgcagttt ca 22

<210> 49
<211> 30
<212> DNA
<213> Artificial
<220>
<223> An artificially synthesized primer sequence for PCR of pcDNA3.1 H 1RNA gene fragment.

<400> 49
tgccgatcca gagcagattt tactgagagt 30

<210> 50
<211> 29
<212> DNA
<213> Artificial
<220>
<223> An artificially synthesized primer sequence for PCR of pcDNA3.1 H 1RNA gene fragment.

<400> 50
ctctatotcg agtgaggcgg aaagaacca 29

<210> 51
<211> 47
<212> DNA
<213> Artificial
<220>
<223> An artificially synthesized primer sequence for PCR of the ligate d DNA.

<400> 51
tttaagcttg aagaccattt ttggaaaaaaaaaaaaaaaaaaaaac 47

<210> 52
<211> 34
<212> DNA
<213> Artificial
<220>
<223> An artificially synthesized primer sequence for PCR of the ligate d DNA.

<400> 52
tttaagcttg aagacatggg aaagagtggc ctc 34

<210> 53
 <211> 5089
 <212> DNA
 <213> Artificial

 <220>
 <223> An artificially synthesized vector sequence.

 <220>
 <221> misc_feature
 <222> (489)..(492)
 <223> " indicates GAP.

 <400> 53

gacggatcgg	gagatctccc	gatcccctat	ggtgtcactct	cagtacaatc	tgtcttggat	60
ccactagtaa	cgcccgccag	tgtgtctggaa	ttccggcttgg	tagccaagtg	caggttatag	120
ggagctgaag	ggaagggggt	cacagttagt	ggcatctttc	ttttctgact	gcccgcccc	180
cgcacatggcgt	cccgcgatat	tgagctccga	acccctctcc	ctggccggcgc	cggtgtctccg	240
tcggccggcgc	gcccgcatgg	aatttcaacg	ctgacgtcat	caacccgctc	caaggaatcg	300
cggcccgagt	gtcacttaggc	ggaaacaccc	agcgccgcgt	cgccctggca	ggaagatggc	360
tgtgaggagc	aggggagtgg	cgccttgc	tatttgcatt	tgcgtatgt	ttctggaaa	420
tcaccataaa	cgtggaaatgt	ctttggattt	ggaaatotta	taagtctgt	atgagaccac	480
tctttccnn	ntttttggg	aaaaaaa	aaaaaaa	aacgaaaccc	ggccggccgc	540
ggtgttca	gcctataatc	ccagcaattt	gggaggccga	ggggggccga	tcacaaggtc	600
aggaggctcga	gaccatccag	gtaaacacgg	tggaaacccc	cccatctct	actaaaaaaa	660
aaaaatacaa	aaaatttagcc	attagccggg	cgtgttggcg	ggcgctata	atccageta	720
cttgggaggc	tgaagcagaa	ttgggtgttac	ccggggaggcg	gacgttgcag	ttggccgaga	780
tcgcgcgcac	tcgcattccag	cctggggcgcac	agagogatc	tcaaaaaaaa	aaccgagtgg	840
aatgtgaaaa	gtccctgtaa	actgcagaaa	cccaagcgcga	attctgcaga	tatccatcac	900
actggccgc	gctcgagtga	ggccggaaaga	accagctggg	gtctctaggg	gtatccccac	960
gcccctgt	gccccgcatt	aagcgcggcg	gggtgttgg	ttacgcgcag	cggtgaccgc	1020
acacttccaa	gcccccttgc	gcccccttct	ttcgctttct	tcccttcctt	tctcgccacg	1080
ttcgcggcgt	ttccctgtca	agctctaaat	ogggggotcc	cttttaggtt	ccgattttatg	1140
gctttacgc	acctcgaccc	aaaaaaactt	gatttaggtt	atgttcaacg	tagtggcca	1200
tgccttgcgt	agacggttt	tgccttttgc	acgttggagt	ccacgttctt	taatagtgg	1260
ctcttgcgttcc	aaactggaa	aaactactcaac	cttatttcgg	totattttt	tgattttataa	1320
gggattttgc	cgatttgcgc	ctattggta	aaaaatgac	tgatttaaca	aaaatttaac	1380
gcaattaat	tctgtggaaat	gtgtgtcagt	taggttgg	aaatccccca	ggctccccag	1440
caggcagaag	tatgaaacgc	atgcatttca	attagtcaac	aaccaggtgt	ggaaagtccc	1500
caggctcccc	agcaggcaga	agttatgc	gcatgcac	caattagtca	gcaaccatag	1560
tcccgcccc	aactccgccc	atcccgcccc	taactccggc	cagttccggc	catttccgc	1620
cccatggct	actaattttt	tttattttatg	cagaggccga	ggccgcctct	gccttgcgc	1680
tattcccgaa	gtatgtggaa	ggcttttttgc	gaggcctagg	cttttgc	aaaagctccccgg	1740
gagcttgcgt	atccatttttgc	ggatgttgc	aagagacagg	atgaggatgc	tttgcgtatg	1800
ttgaaacaaga	ttggatgttgc	gcagggttgc	cggccgcgttgc	gggtggagagg	ctatttgcgt	1860
atgactggc	acaacacaga	atccggctgt	ctgtatgcgc	cgtgttccgg	ctgtcagcgc	1920
aggggcggcc	ggtttgcgtt	gtcaagaccc	acctgtccgg	ttcccttgcgt	gaactgcagg	1980
acgaggccgc	gggggtatcg	ttggccgc	ccacggccgt	tccttgcgc	gctgtgtcg	2040
acgttgcac	tgaagcggga	aggactgtgc	tgctattttgg	cgaatgtccgg	gggcaggatc	2100
tcctgtcata	tcacatttgc	cctggccgaga	aagtatccat	catggctgtat	gcaatgcggc	2160
gggtgtatcac	gttgcgttgc	gttacatgc	catttgcacca	ccaaagcggaaa	catcgatcg	2220
agcgagcag	tactcgatgc	gaagccggc	ttgtcgatca	ggatgtatcg	gacgaagagc	2280
atcagggttgc	cgccgcaccc	gaactgttgc	ccaggctcaa	ggccgcgtat	cccgacggcg	2340
aggatctgt	cggtggccat	ggogatgcgt	gttttgcgt	tatcatgttgc	gaaaatggcc	2400
gttttttgcgt	attcatgcac	ttggccgc	ttgggtgtgg	ggaccgcgtat	caggacatag	2460
cggtggctac	ccgtgtatgc	gttgcgtatgc	ttggccgcgtat	atgggcgtac	cgcttccctcg	2520
tgctttacgg	tatcgccgcgt	cccgatttgc	agcgatcg	cttctatgc	tttgcgt	2580

agttcttctg	agcgggactc	tggggttoga	aatgaccgac	caaggcagc	ccaacctgcc	2640
atcacagat	ttcgattcca	cggccgcctt	ctatgaaagg	ttggggttcg	gaatcgffff	2700
coggagcgc	ggctggatga	tcctccagcg	cgggatctc	atgtggagt	tcttcgccea	2760
ccccaaacttgc	tttattgcag	tttataatgg	ttacaaaataa	agcaatagca	tcacaaaattt	2820
cacaataaaa	gcatttttt	cactgcattc	tagttgttgt	ttgtccaaac	tcatcaatgt	2880
atcttatacat	gtctgtatac	ctgtcgacctc	tagctagac	ttggcgttaat	catggcata	2940
gctgtttcct	gtgtgaattt	tttataccgt	cacaatttca	cacaacatac	gagccggaaag	3000
cataaaagtgt	aaagcctggg	gtgcctaatt	agtggactaa	ctcacattaa	ttgcgttgcg	3060
ctcaactgccc	gtttccagt	cgggaaacct	gtcgtccag	ctgcattaaat	gaatcgccca	3120
acgogogggg	agaggcggtt	tgctgtattgg	gogcttcc	gttccctcgc	tcactgactc	3180
gtgtgcgtcg	gtcttgcgg	tgccggcgac	gttatacgct	cactcaaagg	cgttaatacg	3240
gttattccaca	gaatcagggg	ataacgcagg	aaagaacatg	tgagccaaag	gccagcaaaa	3300
ggccaggaaac	cgtaaaaaagg	ccgcgttgt	ggcgtttttc	cataggctcc	gccccctgaa	3360
cgagcatcac	aaaaatgac	gtcaagtca	gagggtggca	aaccgcacag	gactataaaag	3420
ataccaggcg	ttttccctgt	gaagetcctc	ctgtcgctct	ctgttccg	ccotgcgcgt	3480
tacggatac	ctgtcgccct	ttctcccttc	ggaaagegtg	gogcttctc	atagetcact	3540
ctgttaggtat	ctcaattcgg	ttttaggtgt	tcgttcaag	ctgggtgt	tgcagaacc	3600
ccccgttcag	cccgaccgct	gccccttate	cggttaactat	cgcttgcgt	ccaaacccgt	3660
aagacacgac	ttatcgccac	ttgcagoago	caactggtaac	aggatttagca	gagcgaggta	3720
tgttaggggt	gtcacagagt	tcttgaagtg	gtggcctaac	tacggctaca	ctagaagaac	3780
agtatttggt	atctcgctc	tgctgaagcc	agttacattc	ggaaaaaagag	ttggtagtctc	3840
ttgatccggc	aaacaaaacca	ccgtgttag	cggtttttt	gtttgcaagc	agcagattac	3900
gcccggaaaa	aaaggatctc	aagaagatcc	tttgatctt	totacgggt	ctgacgctca	3960
gtggaaacaa	aactcacgtt	aagggttttt	gttcatgaga	ttatcaaaaa	ggatottcac	4020
ctagatcctt	ttaaattaaa	aatgaagttt	taatcaato	taaagtatat	atagataaaac	4080
tttgtctgac	agtttaccaat	gettaatcag	tgaggcacct	atotcagcga	totgttotatt	4140
tcgttcatcc	atagttgcct	gacttcccg	cgtgtagata	actacgatc	gggaggggctt	4200
accatctggc	cccaagtctg	caatgatacc	gcccggacca	cgctcaccgg	ctccagattt	4260
atcagcaata	aaccagccag	ccggaaaggc	cgagcgcaga	agtggccctg	caacttttac	4320
cgcctccatc	cagtcttata	attgttgcgc	ggaaagctaga	gtaaagtatgt	cgccagttaa	4380
tagtttgcgc	aacgttgcgt	ccattgtac	aggcatctg	gtgtcacgt	cgtcgtttgg	4440
tatggottca	ttcagctccg	gttcccaacg	atcaaggcga	gttacatgt	ccccatgtt	4500
gtgcaaaaaa	ggcggttagct	cottcggctc	tccgatcg	gtcagaagta	agttggccgc	4560
agtgttatca	ctcatggta	tggcagcact	gcataattt	tttactgtca	tgccatccgt	4620
aagatgttt	tctgtactg	gttggacttc	aaccaagtc	ttctgagaat	agtgtatgcg	4680
gcccggcgg	tgctcttgcc	cgccgtcaat	acgggataat	accgcgcac	atagcagaac	4740
ttaaaaatgt	ctcatcattt	gaaaacgttc	ttcggggcga	aaactotoaa	ggatcttacc	4800
gtctgttgcg	tccagttcga	tgttacccac	tgtgcaccc	aactgtatctt	cagcatctt	4860
tacttccacc	agcgtttctg	gttggagcaaa	aacaggaagg	caaaaatgcg	caaaaaagg	4920
aataaggcg	acacggaaat	gttgaatact	catacttctc	cttttcaat	attattgaag	4980
catttatcag	ggttattgtc	tcatgagcgg	atcacatattt	aatgtattt	agaaaaataaa	5040
acaatataggg	gttcccgccca	catttccccc	aaaagtgc	cgtacact		5089

<210> 54
 <211> 51
 <212> DNA
 <213> Artificial

<220>
 <223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 54
 tcccgttagt gtacgaactg gagttcaaga gaotccagg cgtacactaa c 51

<210> 55
 <211> 51
 <212> DNA

<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 55
aaaagttagt gtacgaactg gagtctcttg aactccagtt cgtacactaa c 51

<210> 56
<211> 47
<212> DNA
<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide sequence for hairpin siRNA

<400> 56
gttagtgtac gaactggagt tcaagagact ccagttcgta cactaac 47

<210> 57
<211> 51
<212> DNA
<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 57
tcccggtgtct ctgttggaga tctttcaaga gaagatctcc aacagagaca c 51

<210> 58
<211> 51
<212> DNA
<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 58
aaaagtgtot ctgttggaga tcttctcttg aaagatctcc aacagagaca c 51

<210> 59
<211> 47
<212> DNA
<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide sequence for hairpin siRNA

<400> 59
gtgtctctgt tggagatctt tcaagagaag atctccaaca gagacac 47

<210> 60
<211> 51
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 60
tcccgaaaggc agttgaccaa cacttcaaga gagtgtttgt caactgcctt c 51

<210> 61
<211> 51
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 61
aaaagaaggc agttgaccaa cactctttt aagtgttgtt caactgcctt c 51

<210> 62
<211> 47
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized oligonucleotide sequence for hairpin siRNA

<400> 62
gaaggcagg t gaccaacact tcaagagagt gtttgtcaac tgcctt 47

<210> 63
<211> 51
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 63
tcccgagact gaacagagat gtgttcaaga gacacatctc tggcgtt 51

<210> 64
<211> 51
<212> DNA
<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 64

aaaagagact gaacagagat gtgtctttt aacacatctc tgttcgtctc

51

<210> 65

<211> 47

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide sequence for hairpin siRNA

<400> 65

gagactgaa agagatgtgt tcaagagaca catctctgtt cagtctc

47

<210> 66

<211> 51

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 66

tcccgccaaat gttgtgaggc ttcttcaaga gagaaggctc acaacattgg c

51

<210> 67

<211> 51

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 67

aaaagccaaat gttgtgaggc ttctctcttg aagaaggctc acaacattgg c

51

<210> 68

<211> 47

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide sequence for hairpin siRNA

<400> 68

gcoaatgttg tgaggcttct tcaagagaga agcctcacaa cattggc

47

<210> 69

<211> 51
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 69
tccccctcta cctgtccagc atgttcaaga gacatgtgg acaggttagag g 51

<210> 70
<211> 51
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 70
aaaacctcta cctgtccagc atgtctttt aacatgtgg acaggttagag g 51

<210> 71
<211> 47
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized oligonucleotide sequence for hairpin siRNA

<400> 71
cctctacactg tccagcatgt tcaagagaca tgctggacag gtagagg 47

<210> 72
<211> 51
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 72
tccccgttgtt catcttcgtc atcttcaaga gagatgacga agatgaccag c 51

<210> 73
<211> 51
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 73
aaaagctggc catcttcgtc atctctcttg aagatgacga agatgaccag c 51

<210> 74
<211> 47
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized oligonucleotide sequence for hairpin siRNA

<400> 74
gctggtcatc ttctgtatct tcaagagaga tgacgaagat gaccagc 47

<210> 75
<211> 51
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 75
tcccgttcat cagcgccatc tggttcaaga gaccagatgg cgctgtatgaa c 51

<210> 76
<211> 51
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 76
aaaagttcat cagcgccatc tggttctttg aaccagatgg cgctgtatgaa c 51

<210> 77
<211> 47
<212> DNA
<213> Artificial

<220>
<223> An artificially synthesized oligonucleotide sequence for hairpin siRNA

<400> 77
gttcatcago gccatctggc tcaagagacc agatggcgct gatgaac 47

<210> 78
<211> 51
<212> DNA
<213> Artificial

ONC-PRV0401SQ.txt

<220>

<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 78

tcccggttgtt catacaggta aacttcaaga gagttgacct gtatgacgac c

51

<210> 79

<211> 51

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide sequence for construction of siRNA expression vector.

<400> 79

aaaagggttgtt catacaggta aactctcttg aagttgacct gtatgacgac c

51

<210> 80

<211> 47

<212> DNA

<213> Artificial

<220>

<223> An artificially synthesized oligonucleotide sequence for hairpin siRNA

<400> 80

ggtcgtcata caggtcaact tcaagagagt tgacctgtat gaogacc

47

Application Data Sheet

Application Information

Application number::

Filing Date:: 03/23/04

Application Type:: Provisional

Subject Matter:: Utility

Suggested classification::

Suggested Group Art Unit::

CD-ROM or CD-R??::

Number of CD disks::

Number of copies of CDs::

Sequence Submission:: Yes

Computer Readable Form (CRF)::

Number of copies of CRF::

Title:: METHOD FOR DIAGNOSING NON-SMALL
CELL LUNG CANCER

Attorney Docket Number:: 082368-000500US

Request for Early Publication:: No

Request for Non-Publication:: No

Suggested Drawing Figure:: 11

Total Drawing Sheets:: 11

Small Entity?:: No

Latin name::

Variety denomination name::

Petition included?:: No

Petition Type::

Licensed US Govt. Agency::

Contract or Grant Numbers One::

Secrecy Order in Parent Appl.?:: No

Applicant Information

Applicant Authority Type:: Inventor
Primary Citizenship Country:: Japan
Status:: Full Capacity
Given Name:: Yusuke
Middle Name::
Family Name:: Nakamura
Name Suffix::
City of Residence::
State or Province of Residence:: Kanagawa
Country of Residence:: Japan
Street of Mailing Address::
City of Mailing Address::
State or Province of mailing address:: Kanagawa
Country of mailing address:: Japan
Postal or Zip Code of mailing address::

Applicant Authority Type:: Inventor
Primary Citizenship Country:: Japan
Status:: Full Capacity
Given Name:: Yataro
Middle Name::
Family Name:: Daigo
Name Suffix::
City of Residence::
State or Province of Residence:: Kanagawa
Country of Residence:: Japan
Street of Mailing Address::
City of Mailing Address::
State or Province of mailing address:: Kanagawa
Country of mailing address:: Japan
Postal or Zip Code of mailing address::

Applicant Authority Type:: Inventor
Primary Citizenship Country:: Japan
Status:: Full Capacity
Given Name:: Shuichi
Middle Name::
Family Name:: Nakatsuru
Name Suffix::
City of Residence::
State or Province of Residence:: Saitama
Country of Residence:: Japan
Street of Mailing Address::
City of Mailing Address::
State or Province of mailing address:: Saitama
Country of mailing address:: Japan
Postal or Zip Code of mailing address::

Correspondence Information

Correspondence Customer Number:: 20350

Representative Information

Representative Customer Number:: 20350

Domestic Priority Information

Application:: Continuity Type:: Parent Application:: Parent Filing Date::

Foreign Priority Information

Country:: Application number:: Filing Date::

Assignee Information

Assignee Name::
Street of mailing address::

City of mailing address::

State or Province of mailing address::

Country of mailing address::

Postal or Zip Code of mailing address::

PATENT COOPERATION TREATY
PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

Applicant's or agent's file reference ONC-A0401P	FOR FURTHER ACTION		See item 4 below
International application No. PCT/JP2005/005613	International filing date (<i>day/month/year</i>) 18 March 2005 (18.03.2005)	Priority date (<i>day/month/year</i>) 23 March 2004 (23.03.2004)	
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237			
Applicant ONCOTHERAPY SCIENCE, INC.			

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis.1(a).

2. This REPORT consists of a total of 13 sheets, including this cover sheet.

In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

<input checked="" type="checkbox"/>	Box No. I	Basis of the report
<input checked="" type="checkbox"/>	Box No. II	Priority
<input checked="" type="checkbox"/>	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
<input checked="" type="checkbox"/>	Box No. IV	Lack of unity of invention
<input checked="" type="checkbox"/>	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
<input type="checkbox"/>	Box No. VI	Certain documents cited
<input type="checkbox"/>	Box No. VII	Certain defects in the international application
<input checked="" type="checkbox"/>	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis.2).

<p>The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland</p> <p>Facsimile No. +41 22 338 82 70</p>	<p>Date of issuance of this report 26 September 2006 (26.09.2006)</p>
	<p>Authorized officer Yoshiko Kuwahara e-mail: pt07@wipo.int</p>

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

REC'D 22 DEC 2005

WIPO
PCT

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Applicant's or agent's file reference see form PCT/ISA/220		Date of mailing (day/month/year) see form PCT/ISA/210 (second sheet)	
International application No. PCT/JP2005/005613		International filing date (day/month/year) 18.03.2005	Priority date (day/month/year) 23.03.2004
International Patent Classification (IPC) or both national classification and IPC C12Q1/68			
Applicant ONCOTHERAPY SCIENCE, INC.			

1. This opinion contains indications relating to the following items:

- Box No. I Basis of the opinion
- Box No. II Priority
- Box No. III Non-establishment of opinion with regard to novelty, inventive step and Industrial applicability
- Box No. IV Lack of unity of invention
- Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- Box No. VI Certain documents cited
- Box No. VII Certain defects in the international application
- Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for International preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Guarinos Viñals, E
Telephone No. +49 89 2399-7228



WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/JP2005/005613

Box No. I Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 a sequence listing
 table(s) related to the sequence listing
 - b. format of material:
 in written format
 in computer readable form
 - c. time of filing/furnishing:
 contained in the international application as filed.
 filed together with the international application in computer readable form.
 furnished subsequently to this Authority for the purposes of search.
3. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. II Priority

1. The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43bis.1 and 64.1) is the claimed priority date.
2. This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/JP2005/005613

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

the entire international application,
 claims Nos. 1-30, 32-68

because:

the said international application, or the said claims Nos. 11-25, 35-40 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

the claims, or said claims Nos. 9, 68 when concerning a reagent other than nucleic acid or antibody are so inadequately supported by the description that no meaningful opinion could be formed.

no international search report has been established for the whole application or for said claims Nos. 8, 26, 27, 32, 33, 41, 42, 43, 63, 64, 65 completely and 1-7, 9-18, 28-30, 34-40, 44-62, 66-68 partially when concerning genes GHSR1b, NTSR1, FOXM1 or KOC1.

the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	<input type="checkbox"/> has not been furnished <input type="checkbox"/> does not comply with the standard
the computer readable form	<input type="checkbox"/> has not been furnished <input type="checkbox"/> does not comply with the standard

the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

See separate sheet for further details

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/JP2005/005613

Box No. IV Lack of unity of invention

1. In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:
 - paid additional fees.
 - paid additional fees under protest.
 - not paid additional fees.
2. This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is:
 - complied with
 - not complied with for the following reasons:
see separate sheet
4. Consequently, this report has been established in respect of the following parts of the international application:
 - all parts.
 - the parts relating to claims Nos. 19-25, 31 completely and 1-7, 9-18, 35-40, 44-62, 66-68 partially

**Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, Inventive step or
industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes: Claims	19-25, 31, 35-38, 48-53, 58, 61
	No: Claims	1-7, 9, 10-18, 39, 40, 44-47, 54-55, 57, 59, 60, 62, 66-68
Inventive step (IS)	Yes: Claims	19-25, 31, 38, 58
	No: Claims	35-37, 48-53, 61
Industrial applicability (IA)	Yes: Claims	1-7, 9-10, 31, 44-62, 66-68
	No: Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the International application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/JP2005/005613

Re Item II

Priority

The International Searching Authority has not been able to consider the validity of the priority claim because a copy of the earlier application whose priority has been claimed was not available at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 11-25, as far as an *in vivo* method is concerned, and claims 35-40 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

For the assessment of the present claims 10, 15 22, 28 33 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claims 9 and 68 relate to a reagent defined by reference to a desirable characteristic or property, namely its capacity to detect the expression of the KIF11 gene. The claims cover all products having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/JP2005/005613

result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the reagent as being a nucleic acid or an antibody (as defined in page 24, line 9 of the present application).

Claims 41 and 63 relate to a product defined by reference to a desirable characteristic or property, namely its capacity of being identified by a screening method. The claims cover all products having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for any of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, no search has been carried out for the subject-matter of claims 41 and 63.

Claim 8 relates to a non-small cell lung cancer (NSCLC) reference expression profile that comprises the expression pattern of two or more genes selected from the group consisting of KIF11, GHSR1b, NTSR1 and FOXM1. An expression profile is defined in page 23, lines 28-33 of the present application as the level of expression of these genes. The subject-matter of claim 8 is a mere presentation of information for which no search is required as specified in Rule 39.1(v) PCT. No search was therefore carried out by the International Searching Authority on the subject-matter of claim 8.

Claims relating to inventions in respect of which no international search report has been established need not to be the subject of an international preliminary examination (Rule 66.1(e) PCT). The EPO policy when acting as an International Preliminary Examining Authority is not to carry out a preliminary examination on matter which has not been searched. No examination will therefore be carried out for the subject-matter of claims 8, 41, 63. Examination will be carried out for the subject-matter of claims 9, 68 when the reagent is a nucleic acid or and antibody.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/JP2005/005613

Re Item IV

Lack of unity of invention

The International Searching Authority has found that the present application does not meet the requirements of unity of invention. Five groups of inventions have been identified within the present application (see the International Search Report).

As the applicant has not paid any additional fee the examination will be restricted to the subject-matter for which an International Search Report has been established, i.e. the subject-matter of the first invention. Therefore only the subject-matter of claims 19-25, 31 completely and 1-7, 9-18, 35-40, 44-62, 66-68 partially (when concerning the KIF11 gene) will be examined according to Rule 68.5 PCT.

Re Item V

Reasoned statement with regard to novelty, Inventive step or Industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: US-B1-6 544 766 (BERAUD CHRISTOPHE ET AL) 8 April 2003 (2003-04-08).

D2: US-B1-6 472 521 (UHLMANN EUGEN ET AL) 29 October 2002 (2002-10-29).

D3: WO 03/030832 A (CHIRON CORPORATION; REINHARD, CHRISTOPH; WALTER, ANNETTE) 17 April 2003 (2003-04-17).

D4: WO 03/099224 A (ISIS PHARMACEUTICALS, INC; DOBIE, KENNETH, W; KOLLER, ERICH) 4 December 2003 (2003-12-04).

D5: KAISER ASTRID ET AL: "All-trans-retinoic acid-mediated growth inhibition involves inhibition of human kinesin-related protein HsEg5" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 274, no. 27, 2 July 1999 (1999-07-02), pages 18925-18931.

D6: WEIL D ET AL: "Targeting the kinesin Eg5 to monitor siRNA transfection in mammalian cells." BIOTECHNIQUES, vol. 33, no. 6, December 2002 (2002-12), pages 1244-1248.

D7: SHARP DAVID J ET AL: "The bipolar kinesin, KLP61F, cross-links microtubules within interpolar microtubule bundles of Drosophila embryonic mitotic spindles" JOURNAL OF CELL BIOLOGY, vol. 144, no. 1, 11 January 1999 (1999-01-11), pages 125-138.

D8: BLANGY A ET AL: "Phosphorylation by p34cdc2 regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation in vivo" CELL, CELL PRESS, CAMBRIDGE, MA, US, vol. 83, 29 December 1995 (1995-12-29), pages 1159-1169.

D9: HOULISTON EVELYN ET AL: "The kinesin-related protein Eg5 associates with both interphase and spindle microtubules during Xenopus early development" DEVELOPMENTAL BIOLOGY, vol. 164, no. 1, 1994, pages 147-159.

D10: US-B1-6 331 396 (SILVERMAN ROBERT H ET AL) 18 December 2001 (2001-12-18).

D11: US-B1-6 706 867 (LORENZ MATTHIAS) 16 March 2004 (2004-03-16).

D12: WO 01/31335 A (CYTOKINETICS, INC; WOOD, KENNETH, W; FINER, JEFFREY, T; BERAUD, CHRIST) 3 May 2001 (2001-05-03).

D13: YARROW J C ET AL: "Phenotypic screening of small molecule libraries by high throughput cell imaging." COMBINATORIAL CHEMISTRY AND HIGH THROUGHPUT SCREENING, vol. 6, no. 4, June 2003 (2003-06), pages 279-286.

D14: WO 01/94629 A (AVALON PHARMACEUTICALS) 13 December 2001 (2001-12-13).

Novelty (Art 33(2) PCT)

Document D1 discloses the human kinesin-related protein identified by SEQ ID NO:29 and encoded by SEQ ID NO:28 that corresponds to the KIF11 gene of the present application identified by SEQ ID NO: 2 and encoded by SEQ ID NO:1.

Claims 44, 45, 46, 47 as presently drafted embrace any dsDNA comprising the KIF11 gene, comprising at least 10 contiguous nucleotides from SEQ ID NO:1, comprising from 19 to 25 contiguous nucleotides from SEQ ID NO:1 or comprising SEQ ID NOs: 32, 33 or 34. This includes the dsDNA fragment encoding KIF11 and the vector comprising said dsDNA fragment as disclosed in D1.

The subject-matter of claims 44-47, 54-55, 57 is therefore not new in the light of D1.

For claims directed to a physical entity, non-distinctive characteristics of a particular intended use, are disregarded in determining novelty of the subject matter (see PCT International Search and Preliminary Examination Guidelines, Chapter 12, paragraph 12.05). Consequently "a composition for treating or preventing NSCLC comprising ..." is regarded as "a composition comprising ..." and "a method for identifying/screening for a compound for treating or preventing NSCLC comprising ..." is regarded as "a method for identifying/screening for a compound comprising ..." for the purpose of determining novelty of the subject matter.

Documents D2-D5 disclose a composition comprising an antisense polynucleotide against the KIF11 gene.

The subject-matter of claim 59 is therefore not new in the light of D2-D5.

Document D6 discloses a composition comprising a siRNA against the KIF11 gene.

The subject-matter of claim 60 is therefore not new in the light of D6.

Documents D7-D9 disclose a composition comprising an antibody that binds to a KIF11 polypeptide.

The subject-matter of claim 62 is therefore not new in the light of D7-D9.

Documents D10 and D11 disclose an array comprising a nucleic acid probe that binds to the KIF11 polynucleotide.

The subject-matter of claims 9, 10 and 68 is therefore not new in the light of D7-D9.

Documents D12 and D13 disclose screening methods for antagonists of the KIF11 polynucleotide/polypeptide. Furthermore D12 discloses methods where the test cells are lung cancerous cells and, among them, squamous cell and adenocarcinoma, i.e. a NSCLC cell.

The subject-matter of claims 11-18 is therefore not new in the light of D12 and D13.

Document D14 discloses SEQ ID NOs: 3028-5303 that correspond to genes that are differentially expressed in lung cancers, particularly in adenocarcinoma and squamous cell carcinoma. Both adenocarcinoma and squamous cell carcinoma belong to the non-small cell lung cancer (NSCLC). SEQ ID NO: 3571-3777 represent genes or gene sequences expressed in malignant lung samples that are not expressed at appreciable levels in non-malignant lung cells (see page 18, lines 19-22). Also disclosed in D14 are a method for diagnosing lung adenocarcinoma and squamous cell carcinoma by determining the expression of any of these genes and a method for treating lung adenocarcinoma and squamous cell carcinoma by administering an antibody that binds to any of these genes. SEQ ID NO: 3760 disclosed in D14 corresponds to the KIF11 gene of the present application identified by SEQ ID NO:1 and encoding SEQ ID NO:2.

The subject-matter of claims 1-7, 39, 40, 66, 67 is therefore not new in the light of D14.

Inventive step (Art 33(3) PCT)

D14 discloses a method for treating or preventing NSCLC by administering an antibody that reduces the amount of KIF11 polypeptide.

The difference between D14 and the subject-matter of claim 35 is that the method of claim

35 involves, instead of the use of an antibody, the use of an antisense against the KIF11 polynucleotide.

In the light of D14 the problem solved by the subject-matter of claim 35 is regarded as the provision of an alternative method for treating or preventing NSCLC, the solution being a method for treating or preventing NSCLC by administering an antisense against the KIF11 polynucleotide.

Said solution is not regarded as inventive because an antisense is a known alternative to suppress the expression of a polypeptide, i.e. to reduce the amount of KIF11 polypeptide. Furthermore documents D2- D5 teach antisenses that are effective in suppressing the expression of the KIF11 polypeptide.

Therefore the subject-matter of claim 35 is not inventive in the light of D14 and D2-D5.

The difference between D14 and the subject-matter of claim 36 is that the method of claim 36 involves, instead of the use of an antibody, the use of a siRNA against the KIF11 polynucleotide.

In the light of D14 the problem solved by the subject-matter of claim 36 is regarded as the provision of an alternative method for treating or preventing NSCLC, the solution being a method for treating or preventing NSCLC by administering a siRNA against the KIF11 polynucleotide.

The provision of a siRNA that is effective in reducing the expression of the KIF11 polynucleotide is regarded as inventive because a person skilled in the art has no reasonable expectation of success when trying to provide such a siRNA. However an inventive step can only be acknowledged for those specific siRNAs that have the desired technical effect.

Claims 36, 48-53, 56 as drafted embrace virtually any siRNA and do therefore not meet the requirements of Art 33(3) PCT.

Claims 37 and 61 refer to SEQ ID NOs: 32, 33 and 34 but are not limited structurally (although a siRNA cannot have an infinite length). They do therefore not meet the requirements of Art 33(3) PCT.

Claims 38 and 58 that refer to the specific siRNAs and limit them structurally meet the requirements of Art 33(3) PCT.

The applicant has proven that the KIF11 polypeptide interacts with KOC1 forming a complex involved in mRNA transporting. There is no indication in the prior art neither about

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/JP2005/005613

the interaction of these two polypeptides nor about their role in mRNA transporting. Therefore the subject-matter of claims 19-25 and 31 is inventive.